Vol. X, No. 2 July, 1923

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

PUBLICATIONS COMMITTEE

W. L. BALLS
V. H. BLACKMAN
A. D. IMMS
R. T. LEIPER
G. A. K. MARSHALL

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, MANAGER
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES AND CANADA)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

The Association of Economic Biologists

President

PROF. E. B. POULTON, M.A., D.Sc., LL.D., F.R.S.

Vice-Presidents

PROF. V. H. BLACKMAN, Sc.D., F.R.S.
SIR JOHN RUSSELL, F.R.S.

Hon. Treasurer

A. D. IMMS, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Editors

W. B. BRIERLEY, D.Sc.
D. WARD CUTLER, M.A.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (General and Botanical)

W. B. BRIERLEY, D.Sc.,
Rothamsted Experimental Station,
Harpenden

Hon. Secretary (Zoology)

J. WATERSTON, M.A., D.Sc.,
Nat. Hist. Museum,
South Kensington, S.W. 7

Council

PROF. J. H. ASHWORTH, F.R.S.
W. F. BEWLEY, D.Sc.
PROF. V. H. BLACKMAN, Sc.D., F.R.S.
F. T. BROOKS, M.A.
A. B. BRUCE, M.A.
E. J. BUTLER, D.Sc., C.I.E., M.B.
A. D. COTTON, F.L.S.
T. GOODEY, D.Sc.
W. E. HILEY, M.A.
J. W. MUNRO, D.Sc.
PROF. J. H. PRIESTLEY, D.S.O., B.Sc.
SIR JOHN RUSSELL, F.R.S.

CONTENTS OF VOL. X, No. 2

PAGE

1. I. The Turnip Gall Weevil. *Ceuthorrhynchus Pleurostigma*, Marsh. (*Coleoptera, Curculionidae.*) Part I. Life-History and Bionomics. By P. V. ISAAC, B.A., D.I.C. (With Plate V and 12 Text-figures) 151
2. The Turnip Gall Weevil, *Ceuthorrhynchus Pleurostigma*, Marsh. Part II. Larval Anatomy. By P. V. ISAAC, B.A., D.I.C. (With Plates VI and VII and 19 Text-figures) . . . 171
3. The Turnip Gall Weevil, *Ceuthorrhynchus Pleurostigma*, Marsh. Part III. Control. By P. V. ISAAC, B.A., D.I.C. 190
4. Studies in Bacteriosis. IX. "Streak Disease of Broad Beans." By SYDNEY G. PAINE and MARGARET S. LACEY. (With Plates VIII—X) 194
5. Studies in Bacteriosis. X. "The use of Serum-Agglutination in the Diagnosis of Plant Parasites." By SYDNEY G. PAINE and MARGARET S. LACEY 204
6. On the Relative Importance of certain Common Grasses as Host Plants of *Oscinella frit*, Linn. By NORMAN CUNLIFFE, M.A. (Cantab. et Oxon.) 210
7. Further Contributions to the Biology of Fresh Water Fish. Effect of Various Trade Wastes. By W. RUSHTON, A.R.C.S., D.I.C. (Lond.), M.Sc. (Ill.), F.L.S. 213
8. Effect of Weight of Seed upon the Resulting Crop. By WINIFRED E. BRENCHLEY, D.Sc., F.L.S. (With 5 Text-figures) 223
9. Notes on the Control of "Cutworms" by Poisoned Bait. By J. C. F. FRYER, M.A. and R. STENTON. (With 3 Text-figures) 241
10. Fungi associated with "Die Back" in Stone Fruit Trees. I. By DOROTHY M. CAYLEY. (With Plates XI—XIV) 253
11. On a New Species of Moniezia from the Sheep, *Ovis aries*. By J. R. W. JENKINS, B.Sc. (With Plate XV and 2 Text-figures) 276
12. Studies on the Sex-Ratio and Related Phenomena. (3) Note on the Influence of Size of Litter. By A. S. PARKES, B.A. (Cantab.). (With 1 Text-figure) 287
13. Review 293

VOLUME X JULY, 1923 No. 2

I. THE TURNIP GALL WEEVIL. *CEUTHORRHYNCHUS PLEUROSTIGMA*, MARSH. (*COLEOPTERA, CURCULIONIDAE*)[1]

PART I. LIFE-HISTORY AND BIONOMICS

BY P. V. ISAAC, B.A., D.I.C.

(*Imperial College of Science and Technology, South Kensington.*)

(With Plate V and 12 Text-figures.)

CONTENTS.

	PAGE
Introduction	151
Classification and description	152
Method and range of enquiry	152
Distribution and history	153
Host plants	153
Economic importance	153
Summary of life-history	154
Description of various stages:	
Egg	154
Larva	154
Pupa	156
Adult	156
Secondary sexual characters	157
Bionomics:	
Emergence of adult	157
Protective habits	158
Stridulation	158
Food habits	159
Age at beginning of fertilisation	159
Fertilisation	160
Age at beginning of oviposition	160
Place of oviposition	160
Bionomics (*continued*):	
Selection of plant for oviposition	160
Act of oviposition	160
Fecundity	160
Duration of egg stage	161
Hatching	161
The larva	162
How larva moves and feeds	162
The gall	162
Pupation	163
The cocoon	163
Duration of life of adult weevils	164
Hibernation	165
Life cycle	165
Seasonal appearance	165
The question of races	166
Progress of infestation	166
Dissemination	167
Natural enemies	168
Summary of observations	169
Bibliography	170

INTRODUCTION.

THIS paper embodies certain observations on the life-history and habits of the Turnip Gall Weevil. The investigation was started in August, 1919, at the suggestion of Prof. H. M. Lefroy. A second paper on the anatomy

[1] A grant in aid of publication of this communication has been given by the University of London.

of the larva of this weevil has also been prepared. Work on the metamorphosis and control experiments is in progress. I have to express my gratitude to Prof. Lefroy under whose direction the work is being conducted, and my thanks to Mr J. C. F. Fryer, Entomologist to the Ministry of Agriculture, for valuable suggestions, and to Messrs P. Cragg and W. J. Lobjoit for giving me all facilities on their farms to study the pest and also to the Ministry of Agriculture for a grant towards the expenses of the research.

CLASSIFICATION AND DESCRIPTION.

The Turnip Gall Weevil, *Ceuthorrhynchus Pleurostigma* Marsh (*sulcicollis* Gyll) belongs to the family Curculionidae in the order Coleoptera. The specific name is based on Fowler's *Coleoptera of the British Isles* (3).

A full technical description of the species is to be found in Curtis's *Farm Insects* (2).

METHOD AND RANGE OF ENQUIRY.

The observations recorded here are the results of laboratory and field-work, the former carried on at the Imperial College of Science, South Kensington, and the latter on farms around Osterley Park, Middlesex.

The habits of the pest and the liability to attack of the various host plants, both wild and cultivated, were studied in the field. For general study of the insect, adults were collected from the field and kept in cages outside the laboratory so that they were under very nearly natural conditions. These beetles were found to feed quite well on fresh turnip slices. Galled plants collected in autumn were kept in cages, some exposed to natural conditions and others protected from rain with the soil either quite dry or with only enough moisture around the rootlets to keep the plant healthy. These gave particulars as to development and the influence of climatic conditions.

The minute details of fertilisation, egg-laying and fecundity were obtained by caging under field conditions, imagines reared in field cages and of known history. These were kept either in numbers or when necessary in couples after observation of the first pairing. Young cabbage plants daily supplied to the latter were regularly examined under a binocular microscope for eggs.

Owing to the extreme difficulty of finding the egg, the unlikelihood of an exposed egg hatching and the impracticability of keeping a particular larva under observation while developing without upsetting normal growth, no individual life-cycle could be followed completely through. The necessary facts of the life-cycle are therefore gathered from obser-

vations on a large number of individuals in various stages of growth through partial periods of development.

DISTRIBUTION AND HISTORY.

This weevil has been reported as assuming pest proportions in several countries in Europe, and occurs all over the British Isles.

The earliest record of an entomological nature of this insect is in Kirby and Spence's *Introduction to Entomology*, First edition, 1815 (6), where it is stated that it must be certain weevil larvae "that cause the clubbing of the roots of cabbages and the knot-like galls on turnips." In a later edition of the same work dated 1856 (7), Spence says that he reared out *Curculio pleurostigma* Marsh (now known as *Ceuthorrhynchus pleurostigma*) from the galls on turnips.

Curtis (2), writing in 1859, included this beetle in his *Farm Insects* but did not think they did more damage than affecting the beauty and symmetry of turnip bulbs. Later, Miss E. A. Ormerod (12), in 1881, wrote that the galls may often run together and exhaust the strength of the plant, and Prof. Theobald (15) in 1907, in *The Report on Economic Zoology*, considered the pest as causing incalculable damage all over the country in turnips and swedes.

HOST PLANTS.

During the course of the present investigation various cultivated *Cruciferae* which showed galls were kept under observation. These included the different varieties of *Brassica oleracea*, as cabbage, cauliflower, kale, broccoli and brussels-sprouts, and of *B. campestris*, as turnips and swedes. In every case this one species of weevil was found to be the cause. *Sinapis arvensis* (charlock) is a favourite wild food-plant. Galls have not been noted on other wild crucifers, though many of them, especially those that occur as weeds on farms, were carefully examined.

ECONOMIC IMPORTANCE.

The larvae of the gall-weevil are found below the bark on the root of various cruciferous plants. Their presence checks the natural growth of the host plant and induces it to form galls. Older plants may, apparently, remain vigorous, but when young plants are attacked they become stunted and unhealthy or completely fail to grow. The larva feeds mainly on the cortical cells, and the growth of the gall supplies it with plenty of such material. But the abnormal process of producing the gall must in itself be detrimental to the vitality of the plant. In very young plants there may be present several larvae which on account

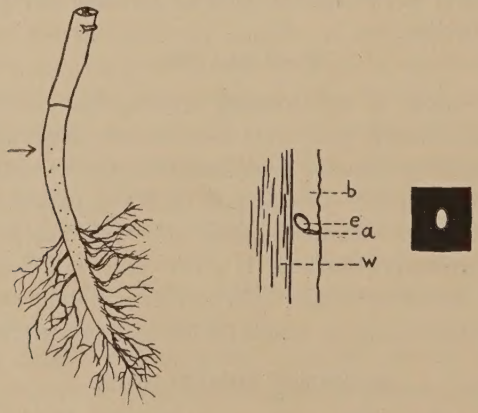
of the small size of the galls will bite into the wood, thereby destroying a large amount of meristematic and fibro-vascular tissues and injuring the plant beyond recovery.

SUMMARY OF LIFE-HISTORY.

The female weevil lays her eggs singly in the bark on the tap-root, bulb or underground portion of the stem of the selected host plant. A legless grub hatches out and feeds on the inner bark. The presence of the larva stimulates the plant to form a gall around the injured spot. The grub lives inside the gall until full-fed and then bores out into the soil and pupates in an earthen cocoon. From the pupa the adult develops and crawls up to the surface to feed and reproduce.

DESCRIPTION OF VARIOUS STAGES.

The egg (Text-figs. 1, 2 and 3) is minute, almost transparent and soft, oval in shape and measures 0·35 mm. in length and 0·25 mm. in breadth. But these measurements may vary slightly and the egg may appear more roundish. The female always lays her egg in a cavity



Text-fig. 1. Root-portion of young cabbage plant; the arrow marks ground-level; the dotted area marks region favoured for oviposition. ($\frac{1}{2}$ nat. size.)
Text-fig. 2. Egg *in situ*. (×8.) *a*, puncture; *b*, bark; *e*, egg; *w*, wood.
Text-fig. 3. Egg. (×8.)

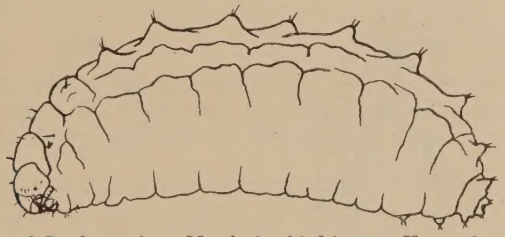
specially drilled in the bark for the purpose, and of a size just large enough to allow the egg to fit in tight. The soft egg thus held under slight pressure adjusts itself to the shape of the cavity.

The larva (Text-fig. 4) is typically curculionid in form, whitish and when full grown about 4 mm. long. The head is small but well exserted.

The mouth parts are hypognathous. Three thoracic and ten abdominal segments are distinct, the last abdominal segment being very minute.

Except for a small fleshy labial area the cephalic region is entirely covered by a well chitinised head-shield on which dorsally the epicranial suture, dividing off anteriorly into the frontal sutures, is clearly marked. The antennae are only represented each by a short two-jointed process close to the base of the mandible at the lateral angle of the frons. There is a distinct dark eye-spot beneath the exoskeleton, on either side.

The body is very much wrinkled transversely. The prothorax has a dorsal thickening into which the head may be slightly withdrawn. The cuticle on this area is minutely punctate. There is a lateral row of tubercles, one on each segment, those on the last abdominal segments being very pronounced, but the others dwindle as they near the head, and there is no tubercle on the prothorax.



Text-fig. 4. Larva of *C. pleurostigma* Marsh, in third instar. Ventro-lateral view showing lateral tubercles. (× 15.)

Spiracles are present on the mesothorax and on the first eight abdominal segments. There are a few minute spines distributed over the body, those on the head, the prothorax and the lateral tubercles being more prominent.

The grubs are usually whitish in colour, but, being rather transparent, those that feed in swedes, owing to the contents of the intestines, may appear reddish-yellow.

The larva moults three times before pupation, and the three instars are separately described below.

First instar. The just-hatched larva is 0·7 mm. long and 0·2 mm. across the head-shield which has in this instar a grey colour.

Second instar. Directly after the first moult the larva is 0·9 mm. long and 0·35 mm. across the head-shield. The whole body as well as the head appears remarkably translucent.

Final instar. At the beginning the larva is 2 mm. long and 0·63 mm. across the head-shield, which is distinctly brownish-yellow and hard in this instar. When ready to pupate the length is nearly 5 mm.

The pupa (Text-figs. 5 and 6) is uniformly white when just transformed and measures 3·7 mm. in length and 2·5 mm. across the broadest part of the body. The head is withdrawn into the prothorax and the rostrum folded on its venter. The antennal scapes run cephalad along the sides of the rostrum, the funicles are directed outward and caudad. Pro- and meso-thoracic legs are folded on the venter and lie over the wing sheaths which are directed on to and press close on the venter. The elytra cover the basal portions of the lower pair, whose exposed distal tips meet or nearly meet on the venter of the abdomen. The third pair of legs are folded below the wings and only the femero-tibial joints of each is exposed, being visible on the lateral aspect of the pupa. Looked at dorsally, the head is only seen to a small extent as it is withdrawn into the collar-like anterior margin of the prothorax. Scattered over the body there are several tubercles bearing short stiff chitinous bristles. These

Text-fig. 5. Pupa of *C. pleurostigma* Marsh, ventral view. (× 10.)
Text-fig. 6. Dorsal view of head and prothorax of pupa. (× 10.)

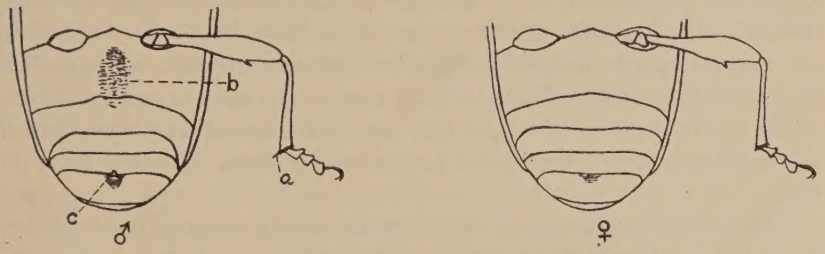
serve to support the pupa in the cocoon, keeping the soft body from contact with the earthen covering. There is one pair of such spines on the vertex, one pair between the eyes, one pair about the middle of the rostrum and one pair on every femero-tibial joint. On the dorsal aspect of the pronotum on either side of a median groove there is a longitudinal row of three and laterad of these, one close to the anterior margin, one where the pronotum broadens out, and a group of three on the prominence at the hind angles of the pronotum. At the posterior extremity of the abdomen there is a pair of very strong terminal hook-like spines.

As development proceeds the dark compound eyes become manifest, and within their respective sheaths the antennal scapes, the claws and the wings form and darken.

The adult. The size of the individual does not vary to any marked extent.

SECONDARY SEXUAL CHARACTERS.

Certain minute external characters which differentiate the sexes may, however, be dealt with here (Text-figs. 7 and 8). The male has a strong spine at the distal extremity of the hind tibiae and a widely impressed median neutral area on the abdomen occupying the apparent first and part of the second sternite; also on the fourth abdominal sternite on its posterior margin a minute median indentation with a short tooth on either side of it. Examination of the abdominal tergite shows that while



Text-figs. 7 and 8. Ventral view (diagrammatic) of abdomen and left hind leg of male (Fig. 7) and female (Fig. 8), to show sexual difference (much magnified). *a*, tibial spine; *b*, impressed area on abdomen; *c*, indentation on fourth sternite.

in the female the pygidial position is occupied by the seventh, in the male the seventh tergite is pro-pygidial, an eighth visible tergite being the pygidial. It may be stated here that the use of the terms pygidium and pro-pygidium, as is often done, without mentioning the numerical relation of the segment, would be confusing. The seventh tergite of the female is thickly chitinised and granular. In the male the seventh and part of the eighth are uniformly thickened and granulate and fit together to occupy an area corresponding to that of the seventh alone in the female.

BIONOMICS.

Emergence of adult. The adult beetle emerges from the cocoon by bursting it. By spreading out the legs and raising its body the beetle secures a firm wedged-in position inside the cocoon. The rostrum, which has till now been more or less closely applied to the venter of the thorax, is now forced outward, causing it to press against the cocoon, until it splits.

This is the normal method of emergence and is only possible under normal conditions when the cell is fairly moist and yielding. But if cocoons are kept dry they become so hard that a large proportion of beetles fail to break through, though they may live for nearly forty days without food in the cocoon. The few that emerge only succeed owing to some accidental flaw in their cocoons.

Pupation takes place among the roots and therefore cocoons are usually found from the surface to about four inches below. Where they were experimentally buried eight inches below soil the imagines emerged.

Directly the beetles come to the surface they seek and take shelter in some dark place under clods, or fallen leaves, or in a crevice in the soil. They will remain here for about 24 hours, attaining the final degree of hardness before they wander about.

Protective habits. These weevils are well adapted to protect themselves from their enemies. Being small and uniformly black dorsally, it is very difficult when they are in the soil to discriminate them. Further, they are very slow, seldom take to flight and generally remain hidden while feeding, resting or travelling. If they happen to be in an exposed position, at the least perception of danger they will relax their hold and drop down. A quickly moving object within a few inches of them will make them do this.

After dropping down and thereby temporarily escaping from danger, it ensures greater safety by turning on its back as soon as it comes to rest, folding the legs close to the body and remaining perfectly motionless. In this attitude the yellowish-grey colour and scaly appearance of the ventral surface, the roughened flattish aspect and the different outline, contribute to produce a picture quite different from the dorsal view of the insect. By remaining motionless the similitude to an irregular particle of some lifeless material is completed. This procedure is usually known as "shamming or feigning death," but it should be understood that it works on the lines of mimicry and that if an insect only shammed to be dead but continued to present any degree of resemblance to its original figure it does not augment the chances of its safety.

Still another means of protection is the power to frighten enemies by producing a quick succession of short screeches.

Stridulation. The sound is produced by protruding and retracting the abdomen rapidly, thereby drawing the apical hard granulate dorsal surface, provided by the seventh tergite in the female and the seventh

Text-figs. 9, 10 and 11. Stridulating mechanism; Fig. 9, ventral view of left elytron; Fig. 10, granulated area ("scraper") in female; Fig. 11, the scraper in the male. (×12.). *F*, file; *S*, scraper.

and eighth in the male, across the minute transverse ridges on the flattened area on the inside of the apical margin of the elytra (Text-figs. 9, 10 and 11).

Both sexes can stridulate, and it has often been observed that when a beetle is picked up between the fingers it will immediately try to produce the squeak. The sound is quite audible when the insect is held close to one's ears. The only other occasion when this sound is produced is when copulation is attempted and then only by the male. After mounting a female the male in trying to copulate throws out and retracts its abdomen a number of times. The sound produced may probably exert some sort of sexual attraction on the female. But as both sexes invariably stridulate when suddenly picked up, it is probable that the function of the sound is mainly protective.

Food habits. The adults feed on leaves, tender stems, the bark on the root, young pods and flowers of turnips and cabbages and on the foliage and flowers of charlock (*Sinapis arvensis*), hedge-mustard (*Sisymbrium officinale*) and a plant allied to the water rocket (*Radicula palustris*).

As mentioned before, the beetles hide away for about 24 hours from the time of emergence and then begin to feed, always trying to remain concealed. In feeding they bite a hole into the substance and then scoop out as much of the inner succulent tissues as may be reached by twisting the rostrum about. In trying to hide they seem to feel safe only when they are tightly wedged in, away from light either in the soil or within curled up leaves. When about 15 days old they become very active for a period of three or four days and climb up plants, specially on a sunny afternoon, to feed on the inflorescence. Next, they conceal themselves in the soil for about two months, living apparently on little or no food. Sexual maturity is developed during this stage in beetles which emerge early in the year. But those that emerge later reach sexual maturity only after passing the following winter. This statement will be made clear when the different broods are dealt with.

The next phase in the life-history is chiefly concerned with reproduction. The beetles, after the period of concealment or hibernation, resume activity, feeding voraciously, specially on leaves and roots of crucifers, the roots of suitable host plants being specially attractive to the female. They copulate repeatedly, either in the soil or on the bushes, often exposing themselves, but usually when above the soil, keeping to the under surface of the leaves, particularly on the margin as the female often goes here for food, carrying or followed by the male.

Age at the beginning of fertilisation. These beetles are not sexually

mature at the time of emergence, and a long time has to elapse before they exhibit reproductive impulses. Females that emerge early in the year, dissected periodically for six weeks from the time of emergence, showed no formation of eggs. Individuals nine weeks old attempted to copulate when a number of them were confined in a small cage. But in nature generally mating starts only a little later, and in the case of the later brood mating starts only after hibernation.

Fertilisation. Mating takes place on the bushes and in the soil, and the male may remain mounted on the female and be carried about by her for about twelve hours; the actual copulation, however, occupies only a few minutes. As is often the case with weevils, copulation is often repeated during the active life of the individuals.

Age at beginning of oviposition. At least five days have to elapse from the time of the first mating before eggs are laid.

Place of oviposition. The egg is always laid singly in the bark of the tap root or bulb of succulent healthy plants over six weeks old. The number of eggs laid in one plant is only limited by the amount of suitable surface available.

Selection of plant for oviposition. Soft, succulent, healthy, easily penetrable bark tissue is what the egg-laying female tries to select for oviposition, and in this respect the bark on the underground root certainly offers most advantages. Among the roots the rapidly swelling tuber of turnips is the favourite, next comes young cabbage plants and then the various other cultivated crucifers. As breeding proceeds and suitable places become rare, freshly formed galls and the bark at the collar region may all have to receive eggs.

Act of oviposition (Plate V, figs. 1 and 2). The female crawls down the stem on to the root and drilling into the bark with its rostrum excavates in the inner bark a small oval chamber of dimensions slightly less than those of the egg. She withdraws her beak now, and turns about, bringing the abdomen over the puncture. By means of the ovipositor the egg is thrust in. With the egg there is also secreted a gummy substance which quickly hardens and forms a covering at the mouth of the chamber protecting and keeping healthy both the egg and the lacerated plant cells.

The method of oviposition is necessary because the egg has to be protected from adverse conditions and provision has to be made for the helpless, just-hatched larva, to find food easily.

Fecundity. A female beetle on dissection shows four egg-tubes. One which had just started ovipositing had 12 well-developed eggs and in

each tube a series of eggs in course of formation. An egg-laying female will lay per day four eggs during the first three or four days, two during the next few days and only one egg later on. After the first month egg-laying becomes irregular. Sunny evenings and warm nights seem to favour oviposition, no eggs being laid when the days are cloudy or rainy. A study of caged pairs leads to the conclusion that a female may lay a total of 60 eggs.

Duration of egg-stage. The egg hatches in five to seven days in summer and in about 17 days in autumn. Eggs laid very late in the year may hibernate. Some of the autumn eggs may hatch when 60 or 70 days old during a spell of warm weather.

Hatching. The egg is nearly transparent when laid, but when about to hatch, the grey head may be made out occupying one end of it.

It has been said that if the egg is laid loose the larva would be unable to work its way out. This is due to the membranous egg covering and the limitations on the working of the larval mandibles which is the only means the larva has of hatching. As pointed out by Prof. H. M. Lefroy (10) in the case of the larva of *Xestobium tessellatum*, the mandibles open out and meet like the two teeth in a nail-extracting pincers. If the surface to be worked into is plane the two teeth will only slip along on closing and will not get a bite. But if with the teeth apart the head can be pressed into the surface the teeth sink a little into the material and can close into a grip. The larva is curled up in a crescentic form inside the egg. By contracting the abdomen and fixing its tip to a point on the egg shell nearer the head the turgidity of the body is increased and considerable pressure acts on the head, pressing it outward. This may be still better explained by considering the effect of the above movements on the body fluid which is normally under high pressure. The contraction of the abdomen displaces from within it and drives into the anterior region a certain amount of fluid. The abdomen has been so pulled up and fixed that it cannot expand and occupy its original position. The body fluid thus made to occupy lesser space than usual will exert great pressure on the body-wall.

The egg-membrane offers another difficulty. Being soft it will behave like a thin sheet of elastic spread on a frame and will stretch and recede when the mandibles try to sink into it. But if the membrane is closely applied to a fairly hard, non-elastic surface then its elasticity has no play and the mandibles can secure a grip. This explains why the mother beetle leaves her egg under a certain amount of pressure.

The larva. The larva has already been described, and it has been stated that it passes through three instars.

How larva moves and feeds. Each larva gives rise to a gall within which it remains feeding on the cortical tissues, seldom biting into the wood. As the gall grows rapidly the chamber inside it, which the larva makes by eating, does not appear very large. In trying to bite off tissues the larva adopts the same method as in hatching out. Having so fixed its abdomen that in its curved position it can get its downwardly directed mouth parts pressed against the plant surface, it will begin to gnaw off tissues, making first a small cavity and then extend it downward into a groove, the greater curving of the body required to do this maintaining the original extra turgidity and thus saving the larva from having constantly to alter its position.

In locomotion also the device is about the same, the lateral tubercles on the body being here of immense help as they serve to keep the body fixed at points other than the anal tip. To make a forward movement the posterior region of the abdomen is contracted and fixed by means of the tip and the tubercles against the wall of the larval chamber and then the contractor muscles are relaxed. Both these actions take place very quickly. Hereby the body-fluid is subjected to high pressure in a reduced body cavity and then allowed to hammer back, upon which it distends the abdomen and, not having spent all its force, sends a pulsation along the line of least resistance which is towards the head. As it passes along the pulsation pushes each segment a little forward, and when it has pushed the head also the body comes to the normal state of expansion and the body-fluid to its usual state of turgidity and equilibrium is restored. In a distending larva it is possible to see one of the middle segments having its posterior region pressed forward by the expanding segment next behind it, and when the pulsation passes into it, itself expanding towards the anterior and then coming to rest. The grub by this contrivance is able to move every time a length equal to the distance it pulls up its abdominal end. It is possible for it to move backward by reversing the process and contracting the anterior thoracic segments first.

The gall. The gall, though separate for each grub, may often occur in clusters and may then coalesce to some extent. A single gall is a smooth rounded knobby growth about 10 mm. across at its base. In a cluster apparently made of four or five galls 10 to 15 grubs may be found, each in its chamber. While in the feeding stage the larva never bites its way close to the epidermis.

The gall in this case is due to some stimulus acting on the cells next to the larva and causing an abundant and rapid formation of cortical cells. According to Kerner's(5) classification of galls, since only a single plant organ is affected in this case and the gall has no natural cavity or opening, it is to be regarded as one of the "simple solid" ones.

As in the case of most galls which arise from ground tissues, the peculiar appearance is brought about by the abnormal quantitative increase in the number of cells in a definite region—which process Küster(9) terms hyperplasia as different from hypertrophy where the individual cells become modified in size and appearance and which is the way most epidermal galls are formed.

The actual stimulant that causes the hyperplasia is not known, and the investigation into its nature affords a delicate and difficult problem.

Pupation (Plate V, fig. 3). After the larva is full fed it waits for a short time, when its body swells very much and becomes full of a clear liquid. On dissection it is found that this appearance is due to the Malpighian tubules becoming distended with a clear secretion. A larva which has reached full growth will leave the gall only when the days are warm, reaching a maximum temperature of over 56° F., and the soil around has received a good supply of rain, and is quite moist. If galled plants are pulled out and kept dry the larvae will remain within and dry up with them. When under artificial conditions the soil outside is kept dry the larva will prepare its exit, leaving only a thin bit of epidermis intact so that it can emerge at the earliest opportunity, and will retire into the gall. But it will not pupate within. In a stalk, left in the field to dry, the larva will show signs of life for several days up to about eight weeks. When infested stalks are pulled out and spread about in the open after every shower of rain several larvae may emerge and this may continue for about 20 days, after which period the bark dries too hard for the larva to bite through. In breeding cages it has been noticed that grubs ready to cocoon if thrown into dry soil will not cocoon but will die in about 20 days, and that if fresh cocoons are kept dry the pupae may form but will die before developing into the imago.

The cocoon (Plate V, fig. 4). As soon as they reach the soil the grubs make a cocoon of mud and a gummy secretion. The cocoons are found close to the roots and sometimes close to the surface.

The gum used by weevil grubs in cocooning has usually been supposed to be a fluid emitted from an opening just above the anus. Recently, F. Knab(8) stated that the fluid is mainly the product of the Malpighian tubules voided through the anus and that certain rectal and surface

glands may also contribute. In the present case the fluid is entirely the product of the Malpighian tubules, which, it has been previously stated, become, before pupation, swollen with a liquid content.

In about six hours from emergence the cocoon is constructed, though some more time is spent in smoothing down the inside. The larva on finding a spot with loose soil secretes a thin clear fluid from the anus and by wriggling and turning about moistens a layer of soil particles around it. The fluid being gummy holds these particles together. Then the larva curves ventrally to the utmost, bringing the head and anal ends together over a minute pellet of earth, moistens it well with the secretion, picks it up between the mandibles and straightening itself fixes it somewhere in the wall around it or where it finds strengthening necessary. The excess fluid is plastered on the inside by spreading it with the frontal region of the head. This goes on until the gummy soil forms around the larva a uniform crust, with a smooth inner surface well covered with a layer of gum. When the gum dries it causes the soil forming the cocoon to hold together more tightly and also forms a brown leathery lining inside.

After completion of the cocoon metamorphosis proceeds, the larva becomes inactive, the eye spots fade and the thoracic region swells, showing distinct conical areas marking the adult's legs.

The larva now moults and the pupal form appears. The skin splits transversely behind the head and from this another split runs caudad along the dorsum. The head-shield splits dorsally along the sutures into two lateral and one anterior piece which are held together by the cuticle on the fleshy labium and carries with it the old chitin of the mouth-parts. The whole cast covering is rolled off in one piece along the venter towards the anal end.

The pupa rests in the cocoon by means of the spines mentioned in the description. For the proper development of the pupa the cocoon has to remain slightly humid. If it gets dry or soaked the pupa will die. The mode of emergence of the adult has been dealt with already.

DURATION OF LIFE OF ADULTS.

Since the weevil continues to oviposit for several days the question of longevity is of decided importance. Adults that emerge in early summer from hibernated larvae continue active throughout the summer and autumn. All these die off in winter.

Beetles which emerge in late summer often live through the winter and the following spring. The length of imaginal life therefore seems

to be nearly seven months when there is no hibernation and about nine months in case the adult hibernates.

HIBERNATION.

The eggs hatch soon after they are laid, but a few laid in late autumn may hibernate and hatch when there is a succession of warm days. Larvae become inactive when temperature falls below 50° F. All the larvae from eggs laid from the last week of August up to the end of the year will hibernate inside the galls, pass the winter and according to the growth they had reached emerge early or late in spring. No pupae have been noted in winter. The adults are active up to 45° F. and hibernate below that temperature.

LIFE CYCLE.

Egg. The egg hatches in summer on the fifth to the seventh day after it was laid, but takes longer in cold weather.

Larva. The period spent in the different instars is very variable. The whole larval period occupies, if there is no hibernation, about ten weeks, and if there is hibernation about five to six months.

Pupa. The so-called pupal period, which may be taken to include the whole time spent within the cocoon, is extremely variable. In summer it may be only about 35 days and in spring about three months, because those that cocoon early in the year do not change into pupa until after about thirty days.

The whole period of development will be at least about four months when the larva does not hibernate, and about eight months when it has to pass through a winter.

SEASONAL APPEARANCE.

There are two distinct, not successive, broods in the year, one which comes out of eggs laid in spring, which may be called the spring brood, and another from eggs laid in late summer and autumn, which may be distinguished as the summer brood.

The spring brood. The beetles of this brood originate from eggs laid in early spring by adults of the previous year's spring brood which have succeeded in hibernating. The egg-laying seems to take place from March to May and the adults emerge towards the end of July and early in August. Breeding takes place almost entirely in roots of charlock (*Sinapis arvensis*). No other host plant has been noted. Though plenty of cultivated crucifers are available at this time they are not attacked. The parents seem to die off by summer. The fresh imagines do not reach sexual maturity till the following spring.

The summer brood. This is the brood which is of direct economic importance. The parent beetles emerge about the beginning of June, feed for a fortnight and then for nearly four weeks hide in the soil. Sexual activity begins about the end of August; the beetles leave their hiding places, feed voraciously, copulate and begin to lay eggs in cultivated crucifers. The adults die off by winter. The eggs generally hatch before winter and the larvae hibernate within the galls in winter. In spring they resume feeding and when full-fed and the soil around has been well moistened by rains leave the galls and pupate in the soil. March and April are the usual periods for pupation. The imagines emerge late in May or early in June.

THE QUESTION OF RACES.

When describing the two broods that appear within one year points were brought out which show that they are racially different; one brood follows the other in time, but in parentage they come from two distinct lines, each line producing one generation in the year. We have, therefore, two distinct races of *C. pleurostigma.*

(i) The spring race, which breeds in spring in charlock, *Sinapis arvensis*, and passes winter in the adult stage.

(ii) The summer race, which breeds in summer and autumn in crops such as turnips and swedes, *Brassica campestris*, and in cabbages and allied plants, varieties of *B. oleracea*, and passes winter in the larval stage.

There are no morphological differences to suggest that these belong to two different species. The chitinous parts of the male genitalia (Text-fig. 12) in individuals of the two races are exactly alike.

PROGRESS OF INFESTATION.

This aspect of the present investigation, being of the greatest moment from the economic standpoint, has been very carefully gone through. Various cultivated crucifers were kept growing throughout the year and wild crucifers were closely watched.

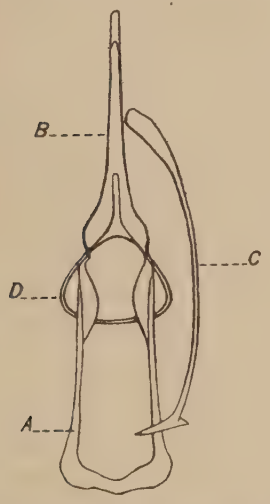
In spring there are only a few ovipositing individuals and since they all belong to the spring race they lay eggs only in charlock, which at this time of the year is in a very flourishing condition. Young brussels-sprouts, cabbages and even turnips are practically left alone at this time.

Those beetles that lay eggs in spring die off before summer, and therefore early in the latter season there are no imagines to lay eggs.

From August to the end of the year eggs are laid exclusively in cultivated crucifers. The large number of adults of the summer race begin

to reproduce during the last quarter of August. From now on, specially during September and early October, crops become rapidly and extensively infested and as a result galled. Spring cabbages in the nursery and turnips are the first favourites. When all these have been nearly gone over older crops are attacked. It has been noticed that when turnips and swedes are grown together, turnips are the first to be badly attacked.

During the year 1919, in September, a count was taken of the progress of infestation in a cabbage nursery at Heston. The percentage of plants



Text-fig. 12. Chitinous parts of male genitalia of *C. pleurostigma* (reared from galls on cabbage). Ventral aspect. *A*, body; *B*, femora; *C*, rod or fork; *D*, ring. (×52.)

with galls on the various dates of examination was as follows: 6th, 30 per cent.; 8th, 30 per cent.; 10th, 55 per cent.; 15th, 66 per cent.; 17th, 70 per cent.; 29th, 78 per cent.

In 1920 on the 13th October, every plant on one square yard of ground in a spring cabbage bed was examined. Out of 164 plants 96 had one or more galls on them, *i.e.* 60 per cent. of the crop was affected, which estimate seems from observation to be well the average. At the same time of the year in a turnip plot measuring half a rod every turnip harboured a few larvae.

DISSEMINATION.

The beetles have not been observed in flight, but they do spread out during the ovipositing season from field to field in search of fresh plants.

When young spring cabbages are taken from the nursery and planted over various plots, since they usually have a number of eggs and larvae in them, it is certain that the operation serves to spread the pest. Further,

this crop and cultural operations connected therewith afford unhampered facilities for the successful breeding of the weevil. Autumn turnips and swedes are consumed before winter and the fate of the larvae in them is doubtful. But spring cabbages carry the larvae through the winter. They are harvested in spring up to the end of April generally. The stalks are either pulled out as soon as the heads have been cut and spread on pasture lands for cattle to feed on or are left in the ground, which is subsequently ploughed up, allowed to dry and then carted away usually late in April to be burned. But the larvae leave the plants in March and April, and provided the infested stalks remain exposed to rain on the soil large numbers of the larvae in them will emerge and pupate.

NATURAL ENEMIES.

The ordinary garden slug, *Limax maxima*, is, to a small extent, a check on the weevil. Though the slug is a vegetable feeder it eats the grub when it feeds on the galls, and slugs have always been observed to have a special liking for the inner tissues of the galls and their habit is to bore right into the gall. While feeding thus they do not show any dislike for the fat whitish weevil grub. The probability is that they develop a taste for them (Plate V, fig. 5).

The larva of *Helophorus rugosus* Ol. (Hydrophilidae) occurs in the soil in certain fields and has been observed to bore into the gall in preference to other parts of the plant, and also to feed on the weevil grubs. The adult is known as the turnip mud beetle. The larva has often been seen to bore right into the gall, casting away the plant tissues bitten out in the process, and thus making a clear effort to get at the weevil larva inside. These larvae are very shy when exposed to light, but more than once they have been taken with the weevil grubs between their mandibles and when kept in cages with galls they burrow and empty the gall of the occupant and also feed on the inner tissues. When there is a cluster of galls they pass from one into another in search of weevil grubs. It may be interesting to note here Fowler's(3) statement that Perris discovered the larva of one of the species of the sub-genus *Empleurus* Hope (to which *H. rugosus* also belongs) in cabbage stalks devouring the larva of *Halitcidae.*

A Braconid, *Diospilus oleraceus* Hal. has been found as an active parasite on the gall-weevil grubs. The female thrusts her egg into the grub through the gall and the larva feeds inside the host. A parasitised grub lives until it emerges and cocoons. The parasite now emerges from the host and the latter dies. It spins a water-tight silken cocoon inside

the host's cocoon, taking care to exclude the dead body of the host. In this cocoon it pupates and the imago bites its way out. The period spent in the cocoon is about a week less than what the host would have in the usual course taken for its metamorphosis. If the host grub is prevented from emerging out of the gall by artificial methods, such as keeping the soil dry, the parasite will after some waiting emerge and cocoon inside the gall, the host dying meanwhile, and the adult will, with its strong mandibles, bite its way out.

The parasite is very active in checking the summer race and large numbers of larvae, especially the earlier ones, are seen to be parasitised, the percentage being about ten for the whole brood. The spring race is not affected by this parasite. The two predators are most active in autumn.

Besides these, various birds peck at the exposed galls and feed on the grubs.

SUMMARY OF OBSERVATIONS.

1. The cause of the smooth round galls on the roots of cultivated crucifers is the presence of the larvae of *Ceuthorrhynchus pleurostigma* Marsh. This pest has a wide distribution over Europe and is common all over the British Isles.

2. The adults feed on the leaves, flowers and young pods of various common cultivated and wild crucifers.

3. The egg is laid in the bark on the root, the larva lives in the gall thrown out by the plant, and the pupa is found in the soil in an earthen cell for constructing which a gummy secretion from the Malpighian tubules is used.

4. There are two distinct races of this insect, a spring race which breeds in *Sinapis arvensis* and not at all in cultivated crucifers, and a summer race which breeds in *Brassica campestris* and *B. oleracea*. The spring race does not injure crops.

5. The summer race begins egg-laying towards the end of August and infests crops. Winter is passed in the larval stage within the galls. Pupation takes place in spring and adults emerge about the beginning of June.

6. If galled plants are kept dry the larvae do not leave the galls but perish inside. If pupation takes place in soil which remains dry several of the pupae die and most of the adults that may develop are unable to break open, and emerge from, the dry pupal cell.

7. The common slug, *Limax maxima*, is fond of the galls, and will often bore right into the galls, feeding on both the plant tissues and the

grubs. Certain birds pick the grubs out of exposed galls. The larva of a Hydrophilid, *Helophorus rugosus*, has similar food habits, and shows a decided preference for the grubs. A Braconid, *Diospilus oleraceus*, is a widespread internal parasite of the weevil grub. When weevil-infested plants with mature grubs are kept dry, though the pest dies within, the parasite, if present, will successfully complete its development, and emerge by biting its way through the dry bark.

BIBLIOGRAPHY.

(1) Connold (1901). *British Vegetable Galls.*
(2) Curtis (1859). *Farm Insects.*
(3) Fowler (1891). *Coleoptera of British Isles.*
(4) Gahan (1900). *Trans. Ent. Soc. London.*
(5) Kerner (1894). *Natural History of Plants.* (Translation by Oliver.)
(6) Kirby and Spence (1815). *An Introduction to Entomology.* (First Edition.)
(7) —— (1856). *An Introduction to Entomology.* (Seventh Edition.)
(8) Knab (1915). *Proc. Ent. Soc. Washington.*
(9) Küster (1903). *Pathological Plant Anatomy.* (Translation by Frances Dorrence.)
(10) Lefroy (1915). Xestobium tessellatum *and roof of Westminster Hall*[1].
(11) Marshall (1916). *Fauna of British India Curculionidae.*
(12) Ormerod (1881). *Manual of Injurious Insects.* (First Edition.)
(13) —— (1890). *Manual of Injurious Insects.* (Second Edition.)
(14) Stanton (1912). *British Plant Galls.*
(15) Theobald (1907). *Report on Economic Zoology.*
(16) *Journal Board of Agriculture* (England). Vol. xxii. 1915.

[1] Not published. MS. Report to Office of Works.

EXPLANATION OF PLATE V

Fig. 1. Puncture made on young cabbage root for oviposition. (×7). Fig. 2. Two weevil eggs exposed by removing part of bark from cabbage root. (×9). Fig. 3. Galls on cabbage root showing exit-holes made by larvae (slightly reduced). Fig. 4. Cocoons made by weevil larvae for pupation. (×4). Fig. 5. Turnip with galls caused by *C. pleurostigma*. Some of the galls have been bored into by the slug *Limax maxima* (slightly reduced). (Fig. 2 photographed by A. M. Alston.)

(*Received February 3rd*, 1922.)

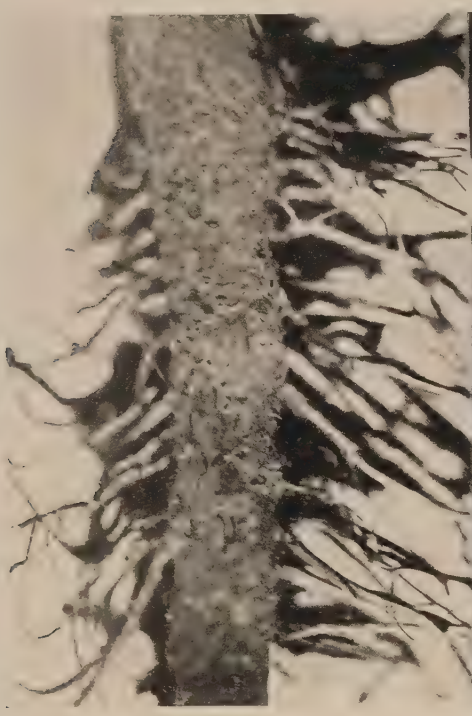


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

ISAAC.—I. The Turnip Gall Weevil. (pp. 151–170.)

THE TURNIP GALL WEEVIL, *CEUTHORRHYNCHUS PLEUROSTIGMA*, MARSH.[1]

PART II. LARVAL ANATOMY

By P. V. ISAAC, B.A., D.I.C.

(*Imperial College of Science and Technology, London.*)

(With Plates VI and VII and 19 Text-figures.)

CONTENTS.

	PAGE		PAGE
I. Material and Methods . .	171	VII. The Circulatory System .	182
II. Exo-Skeleton	172	VI. The Blood Tissue . .	182
III. The Integument . . .	175	IX. The Respiratory System .	183
IV. Endo-skeleton . . .	175	X. The Muscular System .	184
V. The Nervous System . .	175	XI. The Imaginal Buds . .	188
VI. The Digestive System . .	178	XII. Bibliography . . .	188

I. MATERIAL AND METHODS.

Full-grown grubs were collected from galls on cabbage roots, fixed in Carnoy's fluid (formula II), and after 24 hours or more were dehydrated by passing into 90 per cent. alcohol for 24 hours, and thence into absolute alcohol for another 24 hours. For de-alcoholising chloroform was found to be unsatisfactory as the larvae floated on the liquid even after 36 hours, showing that the chloroform had not penetrated thoroughly. Xylol was found to be satisfactory in every way.

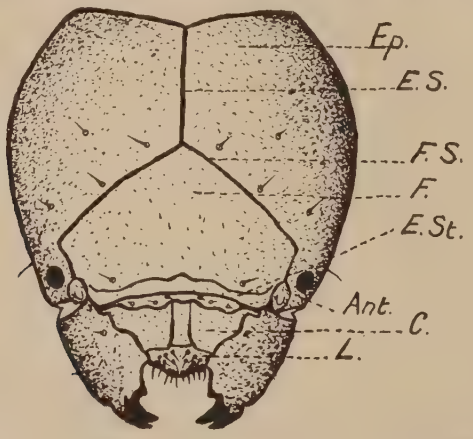
The imbedding was done by taking these grubs saturated in xylol into a mixture of equal parts of xylol and paraffin of 52° C. kept in liquid condition in the oven. After being in this mixture for four hours they were transferred to melted pure paraffin of 52° C. for four hours and were then imbedded in paraffin of 59° F. Sections were prepared in the usual way and were stained in Delafield's Haematoxylin, and either differentiated by washing in acid 70 per cent. alcohol or counterstained with eosin.

[1] A grant in aid of publication of this communication has been received from the University of London.

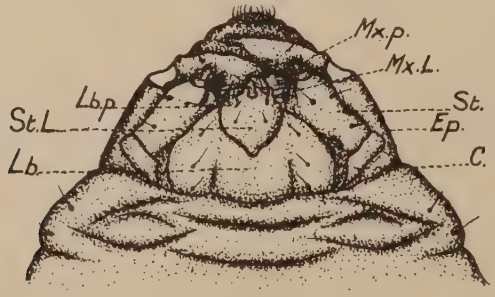
II. EXO-SKELETON.

(1) *The head* (Text-figs. 1 and 2). The head-shield, looked at dorsally, is slightly broader than long; the sides are broadly rounded, the greatest width being near the posterior margin which is angularly emarginate.

From the centre of the emargination along the median line the epicranial suture passes forward dividing the epicranium into two halves.



Text-fig. 1. Dorsal view of head. (×75.) *Ant.*, antenna; *C.*, clypeus; *Ep.*, epicranium; *E.S.*, epicranial suture; *E.St.*, epistoma; *F.*, frons; *F.S.*, frontal suture; *L.*, labrum.



Text-fig. 2. Ventral view of head and prothorax. (×65.) *C.*, cardo; *Ep.*, epicranium; *Lb.*, labium; *Lb.p.*, labial palp; *Mx.L.*, lacinia; *Mx.p.*, maxillary palp; *St.*, stipes; *St.L.*, stipes labii.

This suture before it reaches the middle divides into the two frontal sutures running on either side of the frons to the anterior margin of the head. The frons is sub-cordate and margined along the base by the epistoma and obtuse angulate laterally, the angles being nearly as wide as the one at the apex. The base is widely emarginate along epistoma

for reception of the clypeus, and emarginate at the basal angles for the antenna.

The epicranium curves round to the underside of the head and stops short at the lateral margins of the fleshy maxillo-labium.

In front of the frons, is the broadly transverse clypeus and anterior to it the distally rounded labrum, the clypeus and labrum together forming a sub-triangular plate overlapping the mandibles.

The labrum is transverse, sub-triangular, nearly twice as broad as it is long, and has its sides sinuate. It bears on the dorsal aspect an irregular circle of 20 spines of which ten are marginal, six sub-basal and four sub-apical. On the ventral aspect there are along the apical margin a number of minute tubercles.

The mandibles are rather stout, triangular and tridentate. The apical and median teeth are sub-equal and sharply pointed, the basal one is short and blunt.

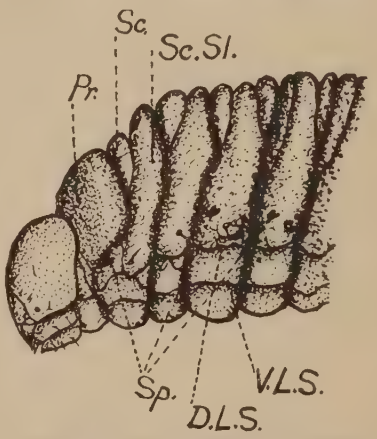
The maxillae are very prominent on the ventral aspect. The cardo is small, the apparent stipes is very large and bears an internal spinose lacinia and an external two-jointed maxillary palp. The labium has a large membranous lobe, consisting of mentum and submentum, contiguous basally with the integument of the prothoracic sternum and laterally with the maxillae. Anterior to this mento-submental lobe and just below the mouth opening is a shield-shaped chitinous area known as the *stipes labii*, bearing on its anterior margin a pair of two-jointed labial palps. On the ventral aspect of the opening of the mouth there is a minute transverse fold, the hypopharynx. The epipharynx has no special outgrowth, being merely a sensitive palatal inner surface of the clypeo-labrum forming the roof of the mouth.

The eyes are extremely rudimentary and are represented by two dark spots on either side of the epicranium close to the base of the mandibles. There is no lens. The antennae also are rudimentary and are found near either basal angle of the frons as small two-jointed processes. The basal segment is very short and flattened, and bears around the base of the small dome-like second segment six very minute spines.

(2) *Spines.* On the head there are a number of spines evidently tactile in function which have a definite arrangement. Their position has been carefully noted. On the basal margin of the frons at each angle close to the indentation for the mandibular condyle, and close to the antenna, there is a single short spine, and posterior to it and closer to the mid region just behind the epistomal suture there is another larger spine. Each lobe of the epicranium bears one spine laterally just behind

the eye and dorsally along the frontal suture a row of three, one close to the epicranial suture, the second more remote but nearer the frontal suture than the other two; a fifth spine occupies the middle position. The clypeus has no spines. The labrum has at its apex a group of 20 hook-like spines whose position has been already described. The mandibles bear three spines, two dorsally and one ventrally, all near the mid-region and close to the outer margin. The maxilla has on its ventral aspect three spines all on the stipes, one close to the cardo and two near the base of the palp. On the labium the stipes labii has one pair occupying a middle position, and on the mento-submental area three pairs, all nearly equidistant, and one behind the other.

(3) *The thorax and abdomen* (Text-fig. 3). The thorax and abdomen bear no appendages and the chitinisation is uniform and smooth except on the dorsum of the prothorax where it is thicker and granulate.



Text-fig. 3. Head and forepart of body. (×25.) *D.L.S.*, dorso-lateral suture; *Pr.*, prothorax; *Sc.*, scutum; *Sc.Sl.*, scuto-scutellum; *Sp.*, spiracle; *V.L.S.*, ventro-lateral suture.

Three thoracic and nine abdominal segments are distinct, and a minute tenth abdominal segment is also to be seen. In freshly moulted specimens two lateral longitudinal folds, the dorso-lateral suture and the ventro-lateral suture, are present dividing each segment into tergal, pleural and sternal areas. The prothoracic tergum is undivided and large. The meso-notum and metanotum have a small scutum and a large scuto-scutellum. The abdominal tergites have their segmentation usually indistinct owing to the swollen condition in which the larva generally is.

At the lateral extremities of all the abdominal tergites just above the dorso-lateral suture, on what may be termed the epipleural lobe, there is a small tubercle bearing a pair of spines of which the anterior one is slightly smaller. The meso- and metathorax do not have these tubercles and have in that region a single spine in place of the pair on the abdomen. The prothoracic epipleural lobes have neither the tubercle nor the spine. A few spines are distributed over other parts of the body.

There are nine pairs of spiracles one on the mesothorax and one on each of the first eight abdominal segments. The abdominal ones are

placed on the epipleural lobes, a little above and anterior to the spine-bearing lateral tubercle. The mesothoracic one is also on the epipleural lobe, above and anterior to the lateral spine already mentioned. But owing to the expansion and thickening of the pronotum, the part of the epipleural lobe which carries this spiracle appears to lie below the prothoracic shield and close to the head, and it has therefore often led to the wrong statement that the only pair of thoracic spiracles present belongs to the prothorax.

III. THE INTEGUMENT.

(Text-fig. 4.)

The integument is made up of an outer chitinous cuticula and an underlying cellular layer. Under high magnification the cuticula appears to be in two strata, an outer homogeneous and dense primary cuticula and a thicker lamellate lower layer, the secondary cuticula. The cellular region shows a thick undifferentiated protoplasmic layer with scattered nuclei—the hypodermis—and below it a uniform hyaline basement membrane.

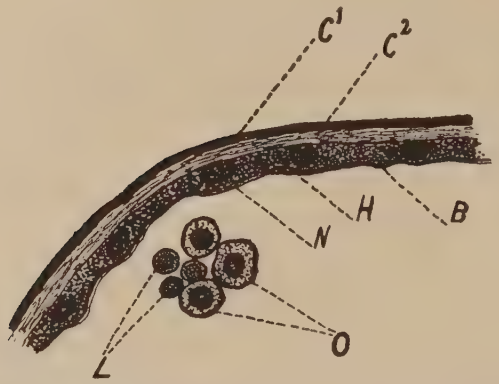
IV. ENDO-SKELETON.

The tentorium (Text-fig. 5). Within the head is a chitinous supporting structure, the tentorium. It consists of a middle transverse plate attached by means of two lateral processes to the ventral margin of the epicranium. Two narrow long anterior and two short posterior arms are also given off. The tentorium lies below the oesophagus. The anterior branches are reflected towards the clypeus and lie on either side of the pharynx. Besides bracing the cranium and holding the cephalic ganglia and oesophagus in place, the tentorium also affords attachment to certain muscles in the head. The central plate is hollow and the whole tentorium is enveloped by a thin epithelial layer resembling the hypodermis.

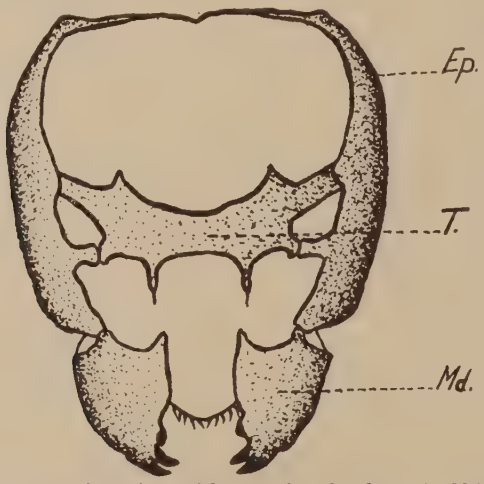
V. THE NERVOUS SYSTEM.

(Text-fig. 6.)

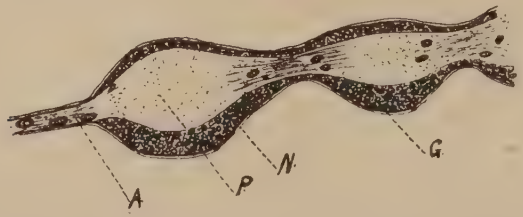
The central nervous system consists of the bilobed supraoesophageal ganglion situated at the back of the head and slightly protruding into the prothorax, connected by oesophageal commissures to the sub-oesophageal ganglion which lies ventrally just above the cuticular fold between the labium and the prosternum, and a double ventral nerve cord with three thoracic and seven abdominal ganglia, the last ganglion being rather elongate and composed of the fusion of a number of ganglia. In transverse section a ganglion exhibits (1) an internal clear medullary portion (Punktsubstanz), and (2) a dense outer layer of ganglion cells, and (3) a thin enveloping membrane, the neurilemma.



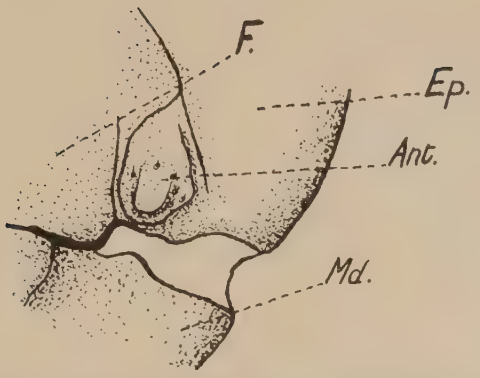
Text-fig. 4. Integument, leucocytes and oenocytes. (×700.) *B.*, basement membrane; C^1, primary cuticula; C^2, secondary cuticula; *H.*, hypodermis; *L.*, leucocyte; *N.*, nucleus; *O.*, oenocyte.



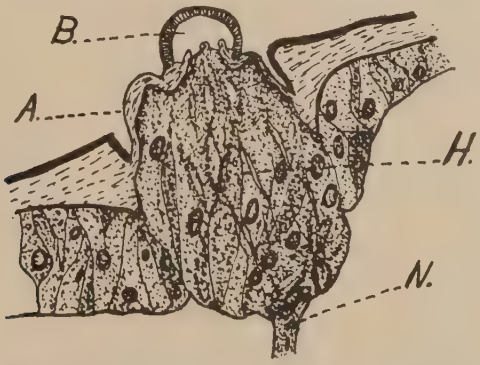
Text-fig. 5. Ventral view of cranium with tentorium in place. (×80.) *Ep.*, epicranium; *Md.*, mandible; *T.*, tentorium.



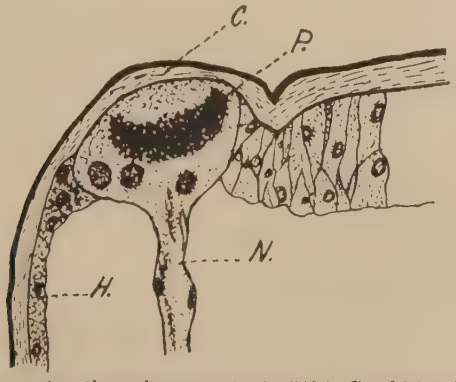
Text-fig. 6. Longitudinal section through the sub-oesophageal ganglion and part of the ventral nerve-cord. (×180.) *A.*, axis cylinder; *G.*, layer of ganglion cells; *N.*, neurilemma; *P.*, "punktsubstanz."



Text-fig. 7. Antenna. (×200.) *Ant.*, antenna; *Ep.*, epicranium; *F.*, frons; *Md.*, mandible.



Text-fig. 8. Median section through antenna. (×600.) *A.*, basal joint of antenna; *B.*, second joint of antenna; *H.*, hypodermal cells; *N.*, nerve connection.



Text-fig. 9. Median section through eye-spot. (×540.) *C.*, chitin; *H.*, hypodermis; *N.* nerve fibre; *P.*, pigment granules.

A nerve-cord consists of a fibrous axis-cylinder with scattered nuclei and the enveloping neurilemma.

The sense-organs noted are the antennae, the eyes, the sensory spines and the olfactory pegs.

The antenna (Text-figs. 7 and 8) has already been described. In a longitudinal section the basal joint appears to have its chitin much thinner than that on the surrounding area. It bulges out only slightly from the head. The second joint is rather dome-shaped and has fine radial striae in the chitin. The eyes (Text-fig. 9) are very little developed and probably are of use only to perceive light. There is no lens. There is only a crowding together of hypodermal cells and a thick concretion of dark pigment granules within them. The cells seem to have their nuclei slightly larger than those in the neighbouring hypodermis and a nerve connection to the eye-spot can also be traced. These eye-spots bear a striking resemblance to the simple optic pits found in certain molluscs.

Text-fig. 10. Maxillary palp. (×500.) *O.P.*, olfactory peg.

The distribution of the chief sensory spines on the head has been already recorded. Besides these there are several other sensory spines on the body. The olfactory pegs (Text-fig. 10) are certain small conical processes on the tips of the maxillary and labial palps. The name has been recently suggested by McIndoo, and is more convenient than the term sensillum styloconicum used by Folsom. The pegs are borne on a very thin chitinous disc. There are eight of them on the maxillary palp and about seven on the labial palp.

VI. THE DIGESTIVE SYSTEM.

The alimentary tract exhibits the three primary regions, the stomodoeum or fore-gut, the mesenteron or mid-gut (stomach) and the proctodoeum or hind-gut. The whole tract measures in a full-grown larva nearly 11 mm. The stomodoeum joins the mesenteron in the second thoracic segment.

There are a pair of short salivary glands. The mesenteron has no gastric coeca. Six Malpighian tubules, of which two are shorter than the rest, are also found.

The alimentary canal has its wall made of four different layers; there is an innermost intima of uniform structure, then a single layer of cellular epithelium, next to it a thin basement membrane and outside all a muscular layer.

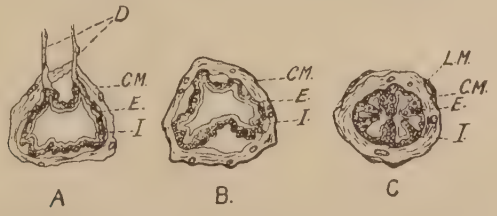
(i) *The stomodoeum.*

(*a*) *General.* The mouth opens between the labrum and the maxillo-labium. Directly behind the mouth the intestine swells a little to form a pharynx. Behind the pharynx is a narrow short oesophagus leading into the next region which has its inner wall thrown into six longitudinal folds. It may be called the proventriculus, since though it is not swollen it corresponds in structure and function to the so-called area in other insects. The stomodoeum now loses the internal ridges, swells a little, forms the oesophageal valve, and then meets the mesenteron. The transformation from stomodoeum to mesenteron is abrupt.

(*b*) The intima is a homogeneous and chitinous layer investing the entire fore-gut, anteriorly contiguous with the chitinous cuticula covering the labium and epipharynx. It forms a sort of loose cover with irregular folds and protects the epithelium below. The stomodoeal epithelium is thin and compact with the nuclei distinct. The basement membrane is very thin and nucleated, and closely envelopes the epithelium. The muscular layer is well developed and is composed almost entirely of circular muscles, but a few longitudinal muscles are also present on the outside of the layer of circular muscles. These muscles evidently, by peristaltic action, push the food from the fore-gut into the stomach.

The Pharynx, Oesophagus, Proventriculus and the Oesophageal Valve.

The Pharynx (Text-fig. 11 A) in transverse section is somewhat triangular in shape with a broad basal area. Along the dorsal aspect the epithelium is folded into a ridge jutting into the lumen. The circular



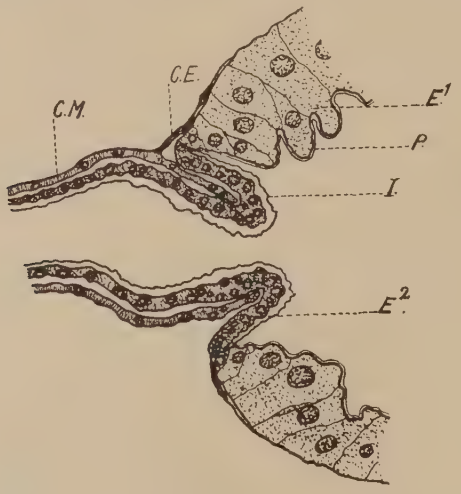
Text-fig. 11. Transverse section through *A*, pharynx; *B.*, oesophagus and *C.*, proventriculus. (×150.) *C.M.*, circular muscles; *D.*, pharyngeal divaricators; *E.*, stomodoeal epithelium; *I.*, intima; *L.M.*, longitudinal muscles.

muscles form a thick covering. Dorsally, certain paired divaricatory muscles running down from the epicranium pierce through the circular muscle layer, and become attached to the epithelium.

The Oesophagus (Text-fig. 11 B). In the oesophagus the epithelium is thrown into irregular waves.

The Proventriculus (Text-fig. 11 c) is slightly narrower than the other region of the stomodoeum and almost circular in section. The epithelium has six definite ridges which project inward so far as nearly to meet. The circular muscles are pronounced and a few longitudinal muscles are also present. The chitinous intima does not exhibit any development of teeth, but the whole structure of this region indicates that its function is to squeeze and strain the food.

The Oesophageal Valve (Text-fig. 12). Just before the stomodoeum joins the mesenteron it runs into the latter and then reflects on itself,



Text-fig. 12. Oesophageal valve. (× 220.) *C.E.*, cardiac extensor; *C.M.*, circular muscles; E^1, epithelium of mesenteron; E^2, epithelium of stomodoeum; *I.*, intima; *P.*, peritrophic membrane.

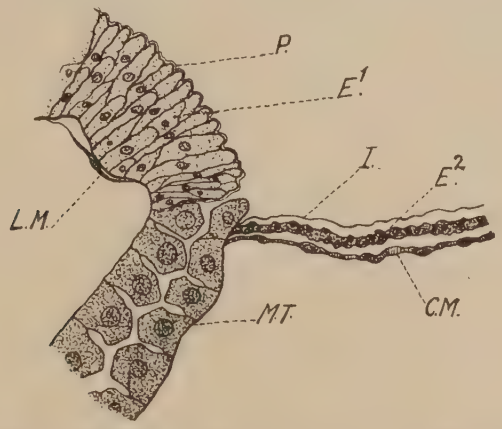
thereby forming a tubular lip which acts as a valve preventing the backward passage of food from the mesenteron. To receive this valve the mesenteron at its anterior end forms a somewhat pear-shaped cavity known as the cardiac chamber. The stomodoeum itself swells a little before the valve to serve as a receiving chamber for food that has to be passed through the valve. The muscular layer is not reflected with the other layers, but ends at the neck where the backward folding starts, but a few longitudinal muscle strands branch off from the stomodoeal layer and run over the cardiac chamber. These may be termed the cardiac extensors. The epithelium shows no change but the cells are more closely arranged in the area of the fold. The chitinous intima retains the appearance of a loose cover. The transition to the mesenteron is clearly marked

by the large columnar epithelial cells of the latter and its closely applied peritrophic membrane.

The action of the valve is probably regulated as follows: masticated and strained food reaches the receiving chamber. The cardiac extensors contract pulling out the sides of the cardiac chamber, thereby increasing its capacity and tending to produce a partial vacuum. This process also allows the tubular lip of the valve to extend out. The peristalsis of the muscles of the stomodoeum drives the food through the valve into the mesenteron. The return of food is prevented by the valve closing automatically when pressure is applied from within the mesenteron.

(ii) *The Mesenteron.* (Text-fig. 13.)

In this region only a few muscle strands are seen, and probably all are circular. The epithelium is made up of large columnar cells. The intima takes the form of a very thin peritrophic membrane closely applied to the epithelium. The Malpighian tubules open into the intestine at the junction of the mesenteron and proctodoeum.



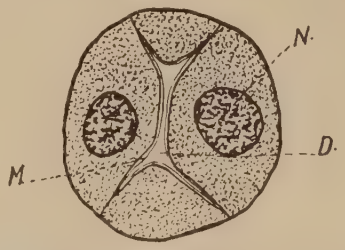
Text-fig. 13. Origin of a Malpighian tubule. (×150.) *C.M.*, circular muscles; E^1, epithelium of mesenteron; E^2, epithelium of proctodoeum; *I.*, intima; *L.M.*, longitudinal muscle; *M.T.*, Malpighian tubule.

(iii) *The Proctodoeum.* (Text-fig. 13.)

In this part the alimentary canal is very narrow and much convoluted. The intima is chitinous and loose and near the oval opening thrown into irregular folds. The epithelial cells are small and closely arranged as in the stomodoeum. The muscular layer is very thick and made up mostly of circular muscles.

The Salivary Glands (Text-fig. 14 and Plate VI, fig. 1). There are two

very minute tubular salivary glands each about 45 mm. long, lying on the ventral aspect below the stomodoeum and the tentorial plate. They open separately on the labium just anterior to the hypopharynx. The epithelial cells of the glands are very large with large nuclei. There is a central canal running the whole length of the tube.



Text-fig. 14. Transverse section through salivary gland. (×approx. 600.)
Text-fig. 15. Transverse section through Malpighian tubule. (×300.) *D.*, duct; *M.*, investing membrane; *N.*, nucleus.

The Malpighian Tubules (Text-fig. 15 and Plate VI, fig. 2). There are six Malpighian tubules, of which two are small and probably do not function. The former in a full-grown larva are nearly 8 mm. long. Each is made up of large polygonal cells. For a length of about 2 mm. at their basal ends these tubules are very narrow, probably this portion serves as a duct. The two small tubules are each about 3 mm. long and along their whole length are as narrow as the ducts of the others. In transverse section a tubule shows a ring of four large cells, protruding into the lumen, and leaving between them a narrow continuous space. A membrane invests the inner free surfaces of the cells.

VII. THE CIRCULATORY SYSTEM.

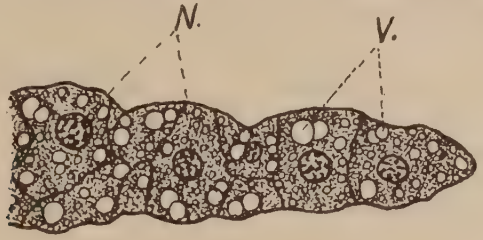
This system seems to consist merely of a simple short tube dorsal to the alimentary tract and extending from the mesothorax to the supra-oesophageal ganglion; just behind the ganglion this tube appears triangular in transverse section with the base above the stomodoeum, but posteriorly it tapers and becomes flattened from side to side and no trace of it is seen in the metathorax. How exactly the dorsal vessel ends anteriorly it has been difficult to note. In structure it has a wall of fine muscle fibres.

VIII. THE BLOOD TISSUE.

(Text-fig. 4.)

Here and there in the plasma small rounded nucleated cells are seen singly or in groups of a few, these are the leucocytes, otherwise known as phagocytes. Besides these certain large cells are also seen free; these are the Oenocytes.

Intimately connected with the blood is the fat body (Text-fig. 16) which occupies a large portion of the body cavity. It has no definite

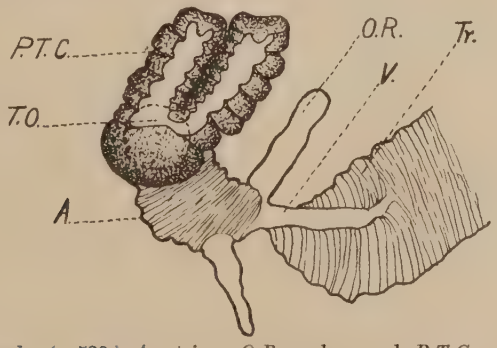


Text-fig. 16. Fat cells. (×430.) *N.*, nucleus; *V.*, vacuoles.

form and more or less surrounds the various internal organs. The individual fat cells are not clearly defined but their nuclei and numerous vacuoles filled with globules of fat are quite clear.

IX. THE RESPIRATORY SYSTEM.

There are two lateral tracheal trunks with numerous ramifications. It has been noted that there are nine pairs of spiracles. The abdominal ones are all nearly equal, but the last two are slightly larger. The mesothoracic one is distinctly larger than the rest.



Text-fig. 17. Spiracle. (×530.) *A.*, atrium; *O.R.*, occlusor rod; *P.T.C.*, peritremal tracheal chamber; *T.O.*, tracheal orifice; *Tr.*, spiracular branch of lateral tracheal trunk; *V.*, vestibule.

The trachea is constructed on the usual lines and has a chitinous lining or intima strengthened on the inner face by thread-like ridges, the taenidia which course in a spiral manner, and an outer layer of tracheal hypodermis.

The spiracles (Text-fig. 17) do not communicate directly with the outside; instead they open into a thin walled U-shaped chamber in the chitin. This chamber has its two arms strengthened by minute transverse

internal ridges. To this chamber the term peritremal tracheal chamber may be applied. A narrow slit runs along the roof of the chamber over both arms and affords communication with the outside air. The lips that guard this slit seem to have the power to come together or open out and thus regulate the vent according to the humidity of the surrounding atmosphere.

A circular opening, the tracheal orifice, leads from the peritremal tracheal chamber into a drum-shaped atrium. The atrium at its inner extremity gives out two short hollow chitinous peg-like processes, the occlusor rods, and then leads into a narrow vestibule which runs on to a side of the spiracular branch of the lateral trunk communicating with it by means of a lateral opening.

The two peg-like processes and the narrow vestibule which is only partially chitinised form the closing or occluding apparatus of the stigmata.

The occlusor rods are connected at their basal ends by very minute occlusor muscles (Plate VI, fig. 3). By their contraction these can bring nearer the two rods and make their bases press against the trachea, and thus regulate or close the opening from the atrium into the vestibule. These muscles consist only of a few cells and are so small that they are distinct only in some of the sections.

In a larva under natural conditions the vent in the peritremal tracheal chamber appears closed and respiration seems to take place directly through the cuticle, below which numerous tracheal branches are found. This adaptation is quite to be expected as the larva is usually living under very moist conditions and while within the gall the method of respiration is certainly akin to that under aquatic conditions. But when the larva is kept in dry open surroundings as in dry soil for a few days and then examined, the vents appear open.

X. THE MUSCULAR SYSTEM.

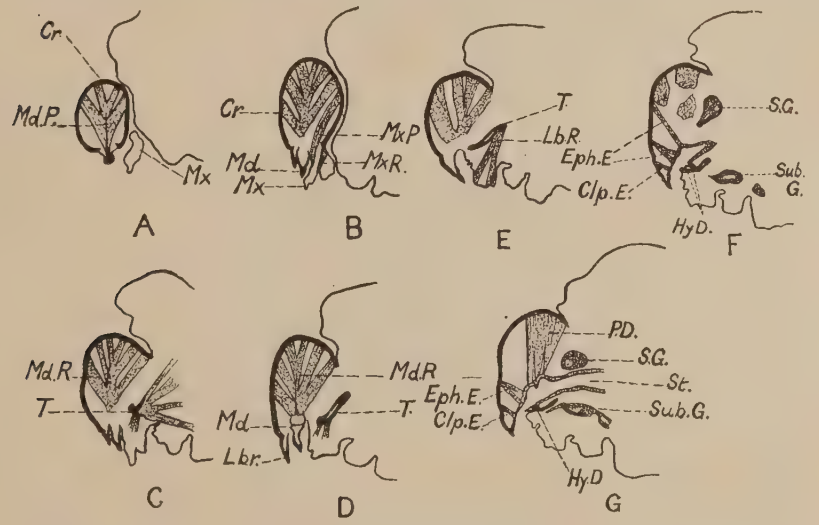
The muscles present a striated appearance and each muscle is a bundle of long fibres with nuclei in different parts and enveloped in a membrane, the sarcolemma.

Besides the muscles connected with the digestive canal and the heart there are three other systems within the body, the cranial muscles controlling the oral appendages, the cephalic retractors and a network of subcutaneous muscles.

The cranial muscles (Text-fig. 18 *A*, *B*, *C*, *D*, *E*, *F* and *G*). The muscles in the head are mainly those controlling the mouth appendages.

In the cavity of the epicranium there is on either side, first the mandibular protractors, and then the mandibular retractors, both very strong sets of muscles attached to the mandible by means of chitinous ligaments from which a thin chitinous plate protrudes into each muscle-set, helping to make the attachment very strong. Ventral to, and almost between these, are the maxillary elevators and below them the maxillary depressors.

In the mid-dorsal region of the cranial cavity, between the mandibular retractors and in front of the supra-oesophageal ganglion are first



Text-fig. 18. *A*, *B*, *C*, *D*, *E*, *F* and *G*. Sagittal sections in series through head and prothorax; *A*., outermost and *G*. nearly median longitudinal section to show arrangement of cranial muscles. (× 28.) *Clp.E.*, elevator of clypeus; *Cr.*, cranium; *Eph.E.*, elevators of epipharynx; *Hy.D.*, depressors of hypopharynx; *Lb.*, labium; *Lbr.*, labrum; *Lb.R.*, retractors of labium; *Md.*, mandible; *Md.P.*, mandibular protractors; *Md.R.*, mandibular retractors; *Mx.*, maxilla; *Mx.P.*, maxillary elevators; *Mx.R.*, maxillary depressors; *P.D.*, pharyngeal divaricators; *S.G.*, supra-oesophageal ganglion; *St.*, stomodoeum; *Sub.G.*, sub-oesophageal ganglion; *T.*, tentorium.

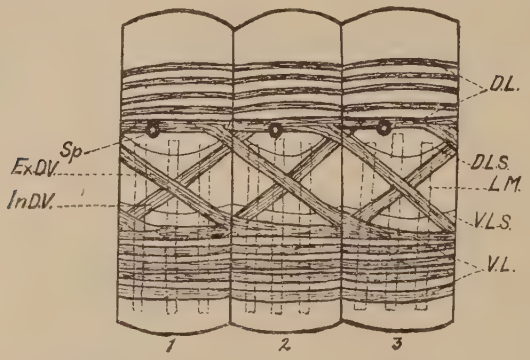
a few thin elevators of the clypeus and then a few elevators of the epipharynx and behind them the paired fan-shaped pharyngeal divaricators.

On either side of the tentorium ventrally from its posterior margin are given out two muscles, one anterior and one posterior, both being apparently retractors of the labium. From the middle of the anterior margin of the tentorium are given out two very short muscles, one above the other, the median depressors of the hypopharynx, their function

being in conjunction with the clypeal and epipharyngeal elevators to open out the mouth aperture.

The cephalic retractors. These are powerful paired muscles in a mid-dorsal position in the prothorax attached anteriorly to the hind margin of the epicranium and posteriorly to the hind region of the prothorax, they draw the head into the prothorax and also elevate it to a small extent.

The subcutaneous muscles (Text-fig. 19). These muscles form a tangled network below the cuticle. The chief muscle-strands seem to be in a regular series repeated in every body segment. They are in two distinct lateral sets, there being practically no muscle strand crossing over from one side to the other, either in the mid-dorsal or mid-ventral line.



Text-fig. 19. Diagrammatic representation of the arrangement of the subcutaneous muscles in the body-segments. 1, 2, 3—three consecutive body-segments, 1 being nearest the head. *D.L.*, dorso-lateral longitudinal muscles; *D.L.S.*, dorso-lateral suture; *Ex.D.V.*, external dorso-ventral oblique muscles; *In.D.V.*, internal dorso-ventral oblique muscles; *L.M.*, lateral muscles; *Sp.*, spiracle; *V.L.*, ventro-lateral longitudinal muscles; *V.L.S.*, ventro-lateral suture.

Just below the hypodermis there are a few slender transverse muscles —the *lateral muscles*. These originate irregularly from points roughly along the dorsal longitudinal fold and are inserted on the ventral wall close to the mid-ventral line. There are three of these on every side in each segment, but they are not all clear in the thorax. The ninth and tenth abdominal segments are too minute to show either these or other muscles. These lateral muscles are of use in drawing closer the dorsal and ventral regions and generally in slight wriggling movements.

Below these are the *external dorso-lateral oblique muscles* and next to them the *internal dorso-lateral oblique muscles*. The external dorso-lateral oblique muscles originate from a point at the junction of two segments,

close to the dorso-lateral suture which marks the junction of the tergal and pleural areas and are inserted at a point in a line with the ventro-lateral suture in the next posterior junction of two segments.

The *internal dorso-lateral oblique muscles* have their points of origin and insertion exactly the reverse of what they are with the externals. So that in the pleural region of every segment these muscles cross one another and form an X. At their points of attachment these muscles do not end abruptly but also send branches, some of which merge into the longitudinals, and others which go further on to the tergal or sternal cuticle. Those to the tergite are fairly well marked, but the others are often indistinct.

There are two sets of longitudinals on either side. These are continuous from the prothorax to the tip of the abdomen. First, there are the *dorso-lateral longitudinal muscles*. They are in very thin strands and are attached to the chitin along the inter-segmental junctions. Then there are the *ventro-lateral longitudinal muscles* forming a very thick strong system. They are also attached to the cuticula along the junctions of the body segments.

In the prothorax the internal dorso-lateral oblique muscles and the ventro-lateral longitudinal muscles originate in the way described, but the point is also one where the ventral margin of the epicranium meets the anterior lateral arms of the tentorium and forms a hard chitinous knob. These muscles are therefore presumably able to retract the head to some extent.

In the terminal abdominal segments certain minor muscle fibres, which go to the tergum from the points of attachment in the dorso-lateral suture, are very well developed.

When the general arrangement of the muscular system has been made out the mechanism of locomotion suggests itself for elucidation. It has been pointed out in the first part of this paper that locomotion in this larva was brought about not entirely and directly by the muscles but indirectly and mainly by muscular contractions in one region of the body increasing the fluid pressure within and transmitting this pressure through the body-fluid to, and thereby causing movements in, other parts of the body. That the maintenance of the turgidity of the body brought about by the general muscular contraction of the body-wall raising the pressure within the body-cavity above that of the atmosphere is an essential factor in effecting many of the so-called muscular movements in all soft-bodied larvae has been suggested by Prof. H. M. Lefroy. The structure of the larva of *C. pleurostigma*, together with observations

on the body movements, provides clear proofs to show that such is the case. A larva when killed by any method loses its turgidity and becomes flaccid. The arrangement of the subcutaneous muscles indicates that their function is to contract the body segments and thus bring about the contraction of a part or the whole of the body. The mouth and anal openings are normally closed due to the pressure of the surrounding body-fluid. The presence of the elevators of the epipharynx, the depressors of the hypopharynx and the pharyngeal divaricators and the thick layer of circular muscles or the coiled proctodoeum, for getting rid of the excreta by peristaltic action, indicate the need to counteract this pressure when necessary.

XI. THE IMAGINAL BUDS.

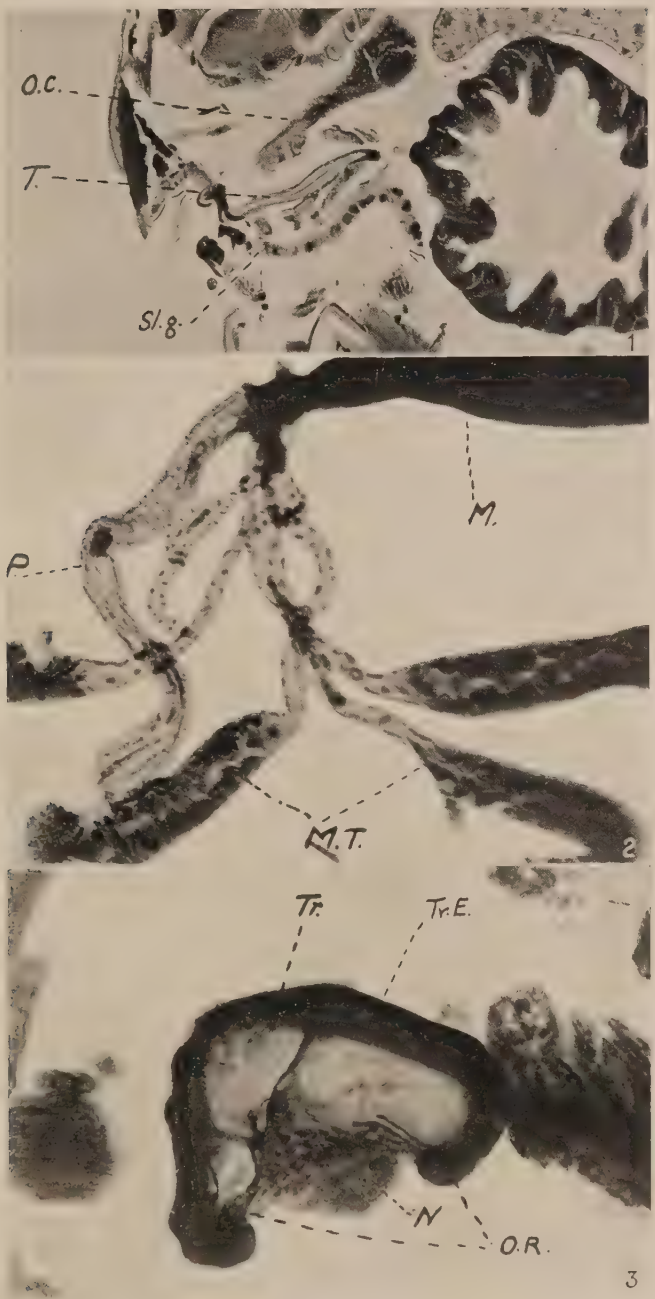
(Plate VII.)

The larvae of holometabolous insects are said to possess certain masses of cells arising from the hypodermis, termed imaginal discs, buds or folds which are the formative centres during metamorphosis of various adult organs.

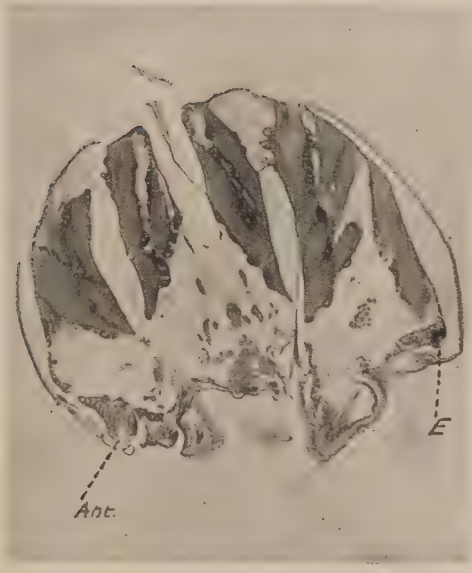
In the full-grown larva of *C. pleurostigma* several such buds are present. Each bud is clearly an evagination of the hypodermis within a cup-shaped cavity formed by the invagination of the hypodermis. There are five pairs of buds distinct, one ventral pair is found in each of the thoracic segments, forming eventually the imaginal legs and one dorsal pair in both the mesothorax and metathorax which grow into the wings. No antennal buds are present and it seems probable that the larval antenna develops into the adult antenna.

XII. BIBLIOGRAPHY.

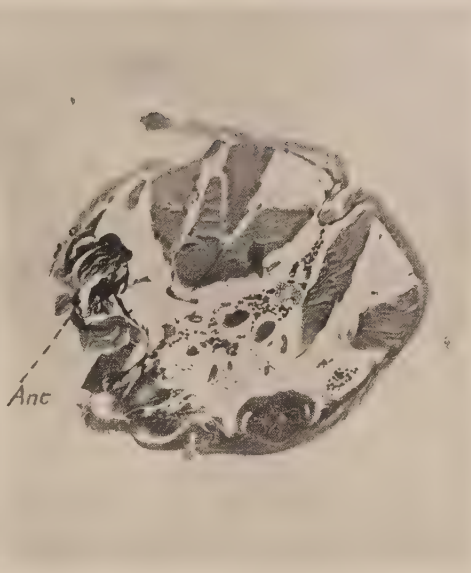
Berlese, A. (1909). *Gli Insetti.*
Folsom, J. W. (1909). *Entomology.*
Gunther (1912). *Zeit. für Wiss. Zool.* c.
Hirschler (1909). *Ibid.* xcii.
Korschellt, E. and Heider, K. (1899). *Text-book of the Embryology of Invertebrates.*
Krancher, O. (1881). *Zeit. für Wiss. Zool.* xxxv.
Macbride, E. W. (1914). *Text-book of Embryology.*
McIndoo, N. E. (1918). *Journal of Morphology,* xxxi.
Packard, A. S. (1909). *A Text-book of Entomology.*
Perris, E. (1877). *Ann. Soc. Linn. Lyon.* xxiii.
Poyarkoff, E. (1910). *Arch. d'anatomie microscopique* (Paris), xii.
Wheeler, W. M. (1899). *Journal of Morphology,* iii.



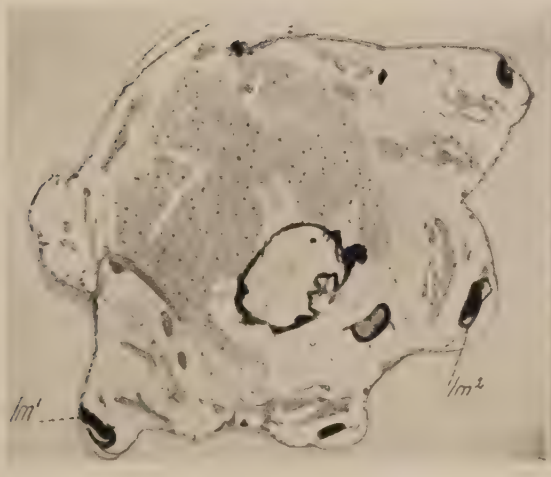
ISAAC.—II. The Turnip Gall Weevil. (pp. 171–189.)



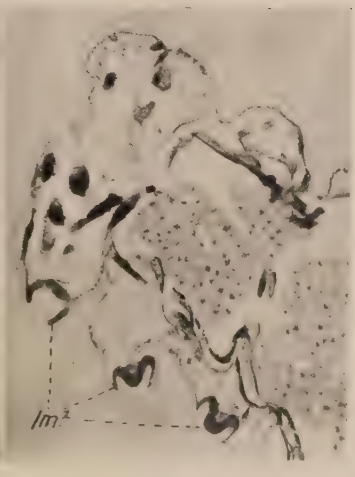
1



2



3



4

ISAAC.—II. The Turnip Gall Weevil. (pp 171–189.)

EXPLANATION OF PLATES VI AND VII

PLATE VI.

Fig. 1. Sagittal section through head and thorax of larva. (× 100.) *O.c.*, oesophageal commissure; *Sl.g.*, salivary gland; *T.*, tentorium.

Fig. 2. The origin of the Malpighian tubules. (× 30.) *M.*, mesenteron; *M.T.*, Malpighian tubule; *P.*, proctodoeum.

Fig. 3. Section through trachea showing occlusor rods and occlusor muscle. (× 800.) *N.*, nucleus of occlusor muscle; *O.R.*, occlusor rod; *Tr.*, trachea; *Tr.E.*, tracheal epithelium.

PLATE VII.

Fig. 1. Transverse section through the head of a larva. (× 90.) *Ant.*, antenna; *E.*, eye.

Fig. 2. Transverse section through nearly the same region as in Fig. 1, in a larva about to pupate. (× 100.)

Fig. 3. Transverse section through the mesothorax of a larva. (× 70.) Im^1, imaginal bud of wing; Im^2, imaginal bud of leg.

Fig. 4. Sagittal section through the thorax of a larva showing the imaginal buds of the legs on one side. (× 75.)

(*Received February 3rd*, 1922.)

THE TURNIP GALL WEEVIL, *CEUTHORRHYNCHUS PLEUROSTIGMA*, MARSH.[1]

PART III. CONTROL

By P. V. ISAAC, B.A., D.I.C.

(*Imperial College of Science and Technology, South Kensington.*)

CONTENTS.

		PAGE
I.	Introduction	190
II.	Soil fumigants	190
III.	Hot water treatment of seedlings	191
IV.	Disposal of old stalks	192
V.	Recommendations	192
VI.	Bibliography	193

I. INTRODUCTION.

The control experiments included the use of soil fumigants to kill the beetles or deter them from ovipositing, dipping cabbage seedlings in hot water to kill the grubs that may be present in the roots, and the proper disposal of old stalks. As the beetles feed on the inner tissues of leaves and flowers and owing to the nature of the crops to be protected, the use of stomach poisons was barred and for obvious reasons no method that would have entailed much expense was attempted.

II. SOIL FUMIGANTS.

For the trial of soil fumigants six plots of spring cabbages, each one rod in area, were selected. These had been sown about the middle of July, 1920. The application of the fumigants was made between the 11th and 14th of September, when the plants had reached a stage likely to be infested. The substances tried were "tar product," naphthalene, dichlorbenzene, active creosote and chlorcresol. One plot was kept for control.

1. "Tar product." This is a powder consisting of ground neutral residue and chalk and 1 per cent. of neutral tar oil. The powder was mixed with nine times its weight of chalk-powder and 5 lbs. of the mixture was spread on one plot.

[1] A grant in aid of publication of this communication has been received from the University of London.

2. Naphthalene. Ordinary naphthalene was used with chalk in the same strength and the same amount as the "tar product."

3. Dichlorbenzene. This is a pleasant smelling crystalline powder which evaporates very slowly. This also was applied to the soil in the same manner and strength as the two previous substances.

4. Active creosote. This was mixed with chalk-powder at the rate of $4\frac{1}{2}$ fl. ozs. to $4\frac{1}{2}$ lbs. and spread over the plot.

5. Chlorortho-cresol. This was used in the liquid state with chalk-powder in the same strength and amount as the active creosote.

On October 18th the plots were examined for results of the treatment. From each plot 200 plants were taken out at random and a count made of the number of galled ones. The result was: "tar product," 139; naphthalene, 129; dichlorbenzene, 124; active creosote, 118; chlorcresol, 112; and in the untreated control plot, 131. This indicates that chlorcresol gave the best results, but compared with the control plot, even this did not protect the plants to any satisfactory extent and the entire experiment shows that the use of repellents on a field scale is not likely to be efficacious.

III. HOT WATER TREATMENT OF SEEDLINGS.

Provided it killed the larvae but did not injure the plant, the immersion of the roots of infested cabbages at the time of transplanting in hot water for a fixed time appeared to provide a simple and inexpensive method for the partial control of the weevil. To get a preliminary idea of the effect of such treatment a number of plants were dipped for a fixed time in water kept at a uniform temperature. After five minutes in water at 107·6° F. the larvae were alive, so were they after 10 minutes immersion, but after 15 minutes in the bath they appeared dead and when examined a few hours after the treatment had revived. At 104·9° F. after 30 minutes the larvae were not dead. At 118·4° F. for 5 minutes the larvae appeared dead but were found to have revived the next day. It was therefore decided to try a long immersion at 107·6° F. and a few bundles of plants were treated for 25, 30, and 35 minutes respectively. After 16 hours from the treatment the larvae in the first set resumed activity, but those in the others were found quite dead. Seedlings from the last two bundles were planted out but all wilted away.

It was next attempted to try a higher temperature for a short time. Immersion at 122° F. for 3 and 2 minutes killed the larvae, but plants so treated failed to grow. It is, therefore, to be concluded that the hot water treatment is a failure.

IV. DISPOSAL OF OLD STALKS.

The next point attended to was the disposal of old stalks in ways that would (1) help the Braconid parasite, *Diospilus oleraceus*, to breed out, or (2) prevent the grubs in the galls from reaching maturity. It has been stated that if galled stems are pulled out and allowed to dry under shelter the grubs perish within, also that if infested plants are kept in soil with only a little moisture and protected from rain, the parasite may cocoon in the gall and emerge while the weevil grubs would remain in the galls and die ultimately. To store old stalks under these conditions is an impossibility and therefore experiments along that line had to be given up.

It was found that if old stalks with larvae in them are stacked up in very large heaps the multiplication of the pest may be checked. Such stacking has to be done as early in spring as possible. In a large heap stacked loose the stalks dry quickly, the top ones to a large extent prevent rainwater from wetting those below from which therefore the grubs will not emerge and they themselves being well exposed dry quickly and are also visited by various birds which pick out the grubs.

V. RECOMMENDATIONS.

Taschenberg (5) recommended the rooting out and burning of infested stalk where possible before the larvae leave the galls. He did not think the application of soot or lime or any other such material of any effect in controlling the weevil attack. Miss E. A. Ormerod (3) suggested the burning of old infested stalks, and not to bury them as this will not prevent the grubs from turning into imagines, or throwing them into farm-yards where the grubs will be choked in wet manure or shooting them into field-ponds away from houses. She also recommended in the case of seedlings the rejection of galled ones or the scraping off of the galls, as a preventive the application of gas lime or soot or fresh earth to the soil. Ritzema Bos (4) indicated the rooting out and burning of galled plants as the only possible check. Prof. Theobald (6) gives very useful advice when he recommends that land after an attack should be deeply ploughed and well rolled and that no crop liable to attack should be put in directly afterwards. But this last recommendation needs explaining in the light of the fact that this weevil does not infest successive crops but breeds in cruciferous crops during one particular season in the year. His suggestion to plough in deep infested stalks may not be altogether effective as it has been noted that grubs in stalks buried 8 ins. deep are able to transform.

The recommendations that are suggested as a result of the present investigation are:

1. Root out all over-wintered infested stalks of crops like brussels sprouts, kales and cabbages, by the beginning of March and of spring cabbages as early as possible, shake off the earth from the roots and stack them up in large heaps and when dry burn them. Never leave infested stalks longer than can be helped in the soil or when they have been pulled out, scattered about, or in small lots.

2. Plough the land deep immediately the infested crop has been removed. This will crush and destroy a large number of cocoons with the pupae within.

3. Avoid in the next autumn a crop liable to attack. It is not necessary to avoid such a crop in spring or early summer as the pest does not attack cultivated crucifers at this time, but only crops grown from about the middle of July and through the autumn. At this time the land should be put under some crop that is immune.

4. Destroy all charlock (*Sinapis arvensis*) and hedge-mustard (*Sisymbrium officinale*) as the adult beetles feed on the foliage and flowers of these. The race of *C. pleurostigma*, which breeds in spring in charlock, is not infectious to cultivated crucifers, but it is possible that some of the beetles emerging very early in the year may attack in autumn some of the crops; besides it is known that charlock is a host of the fungus *Plasmodiophora Brassica* Wor. which causes the "club-root" in many crucifers.

VI. BIBLIOGRAPHY.

(1) Curtis, J. (1859). *Farm Insects.*
(2) Massee, G. (1915). *Diseases of cultivated plants and trees.*
(3) Ormerod, E. A. (1881). *Manual of injurious insects.*
(4) Ritzema Bos, J. (1891). *Tierische Schädliche und Nützliche.*
(5) Taschenberg, E. L. (1879). *Praktische Insekten-Kunde.*
6) Theobald, F. V. (1907). *Report on Economic Zoology.*

(*Received February 3rd*, 1922.)

STUDIES IN BACTERIOSIS. IX

"STREAK DISEASE OF BROAD BEANS"

By SYDNEY G. PAINE and MARGARET S. LACEY.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

(With Plates VIII—X.)

Symptoms of the Disease.

This disease is characterised by chocolate spots on the leaves, broad sunken chocolate streaks with a bronze lustre on the stems, and similar coloured patches on the pods. The leaf spots are often very numerous and cover a large area of the leaf (Pl. VIII, fig. 1); the stem lesions are of varying size from half an inch to four or five inches, they often extend the length of an internode and run intermittently throughout the length of the stem, recalling very strikingly the stem lesions in "stripe" of tomatoes and "streak" in potatoes and sweet peas. The trouble often starts at the base of the plant and works upwards, but sometimes the whole plant seems to be attacked suddenly and uniformly; in the former case soil or seed infection, and in the latter air infection, would seem to be indicated. The lower leaves fall prematurely and the stems are left with a small number of unhealthy leaves at the top with only a few pods of small size and these often much spotted.

Occurrence of the Disease.

The disease was very troublesome upon field beans during the summer of 1920. It commenced in May in South Wales and rapidly spread, or seemed to spread, eastward. It was observed by one of the authors in Devon during June, in Sussex in July, and was reported successively from Buckinghamshire, Cambridgeshire and Lincolnshire. It was undoubtedly very general throughout a large part of England and the evidence of an easterly spread, which would indicate wind dispersal, is not sufficiently strong to be considered reliable. When this investigation began about the middle of June the disease was well advanced, having appeared in Carmarthen some six or eight weeks earlier. In one case,

beans planted in October, 1919, first showed signs of disease on May 25th, small purplish brown spots being observed on the leaves of plants then about five feet high. When next observed, June 8th, the plants were largely defoliated, the remaining leaves showing a good deal of blackening. The weather at the time of the onset was said to have been hot and thundery, and when these fields were examined on July 10th by Mr Wiltshire of the Long Ashton Experiment Station, the canes in the central portion of the field were beaten down by rain, and all the leaves were defoliated except a bunch at the top of each stalk. The whole crop was rapidly destroyed by *Botrytis* which, in all cases, seems to follow closely upon the streak disease.

In many instances it was observed that the plants in the outer portions of the fields were less severely attacked than those in the centre; being more exposed the outer portions would naturally tend to be under drier conditions.

On a farm near Sidmouth in South Devon the onset of the disease occurred during three days of dense fog; in this case the whole field suffered equally and in three weeks the plants were practically stripped of their leaves.

It is probable that the disease is always present to some slight extent upon winter beans and that unfavourable weather conditions were responsible for the extensive epidemic of 1920. One farmer could recall a similar destruction of his beans some thirty years ago and that he had at that time attributed the trouble to lightning injury. Undoubtedly heavy moisture, rain or fog, and high temperature are the conditions which favour the spread of this disease. The freedom from the disease exhibited by the crops of the abnormally dry summer of 1921 is strong evidence in this connection; the disease did, in fact, make its appearance in a field near Bristol which had borne a badly diseased crop the previous year but the plants spontaneously recovered and later not a trace of the disease was to be found.

The losses incurred during 1920 were very heavy, on many farms the crops were not considered worth harvesting, on others the estimated loss was fully 75 per cent. It is probably a low estimate to give the total loss for southern England as 40 per cent. or 50 per cent.

PATHOLOGICAL ANATOMY.

Microscopical examination of the lesions or streaks on the stem shows in many cases destruction of the epidermal and the sub-epidermal cells of the cortex, no effect being found in the deeper tissues. In other cases

the pith is browned for a considerable distance up the stem, and from the pith the disease can be traced by way of the medullary rays to the cortex, and through this it passes to produce lesions under the epidermis as stated above. The spots on the leaves extend through the entire thickness of the leaf or may be confined to the epidermal and sub-epidermal cells only, or, again, only the guard cells of the stomata are found to be discoloured. The first sign of disease of a palisade cell is a reddening of the cell contents, next these appear to be in a state of disorganisation and finally to be reduced to a disintegrated mass held together by a reddish-brown gummy substance. The cell walls become swollen, the middle lamella partly dissolve and the cells collapse. The walls and the granular gummy contents stain strongly with carbol-fuchsin, an effect which is very commonly produced by bacterial action.

The causal organism.

Attempts to isolate a bacterial parasite were not always successful, many of the lesions seemed to contain no living organism, others gave only the usual soil saprophytes, but in about 20 per cent. of all such attempts a yellow organism appeared which proved to be *Bacillus lathyri*.

The symptoms of the disease fully accord with the attack of this organism on other hosts. It has been shown by Manns and Taubenhaus (1), Manns (2), and recently confirmed by Paine and Bewley (3), to produce streak disease of sweet peas and other legumes. The symptoms in these cases and in the stripe disease of tomato (4), which is also caused by *B. lathyri*, are exactly the same as far as stem lesions are concerned as those described above.

B. lathyri was isolated from leaves of beans from Carmarthen, Sidmouth and Bristol. Attempts to obtain the organism from the brown pith of diseased stems and petioles failed in every case. It was also obtained from stem lesions of clover plants from a field near Bristol adjacent to a badly attacked crop of beans.

Along with *B. lathyri* a number of yellow organisms were isolated and many of these appeared to be so similar to it, yet differing in pathogenicity and certain other characters, that a special study of them was made in order to determine whether or no they were non-virulent strains of the same organism. The results of this investigation are contained in a separate paper (5).

INOCULATION EXPERIMENTS.

Preliminary infection experiments made in June, 1920, showed that the disease could be reproduced by grinding up some of the spotted leaves in water and pricking this suspension into healthy bean leaves. In five days the tissue round the point of infection had a typical chocolate brown colour to a depth of 1–2 mm.; the controls were all negative. Following these, however, all experiments conducted during the summer of 1920 were spoiled by an extraordinary prevalence of the disease making it impossible to keep the control plants uninfected.

In April, 1921, an extended series of hypodermic needle infections was made with three strains of *B. lathyri* (one from tomato and two strains 33 and 45 c isolated from beans) and with two strains of a yellow organism (designated 20 and "b") which gave all the reactions of *Pseudomonas Phaseoli*, and which was frequently isolated from diseased plants along with *B. lathyri*. Three plants were inoculated with each of these five strains and sets of three were inoculated with mixtures of the *B. lathyri* strains and the strains 20 and "b" in all possible combinations to see whether this organism had any part in the etiology of the disease. Twelve control plants were inoculated with boiled suspensions of the various organisms. All the plants were cut longitudinally through the point of inoculation at the end of three weeks. The controls showed no sign of disease and the wound made by the needle was only just perceptible.

B. lathyri (tomato strain). Plant (1) red spot, 3 mm. diameter round the hole; vascular bundles brown for a length of 3 inches.

Plant (2), slight brown mark ½ inch up the stem. No interior lesion.

Plant (3), no disease.

B. lathyri (strain 45 c from *Vicia*). Plant (1), brown streak running up the v.b. for 4 inches then passing out through the cortex and causing a reddish-brown streak on the epidermis 2 inches long, ending at the petiole above.

Plant (2), same as (1).

Plant (3), brown streak passing up the v.b. and out through the cortex to produce exterior brown streaks intermittently up the stem. The brown v.b. traceable to a petiole 9 inches up the stem, petiole and leaves spotted with red-brown spots; leaves on the other side of plant not spotted.

B. lathyri (strain 33 from *Vicia*). Plants (1) and (2) show typical red sunken marks 3 mm. diameter on the exterior at the point of inoculation. The v.b. is brown 6 inches up the stem and traceable to angular red streaks on the epidermis as above.

Plant (3) shows very little effect in the stem (very woody stem) but the v.b. is brown 4 inches up and traceable to the petiole above.

Strains 20 and "b." All six plants gave negative results, and the plants inoculated with mixtures of these with *B. lathyri* showed no more extended disease than those with *B. lathyri* alone.

Other inoculation experiments were made with combinations of *B. lathyri* and various white organisms which appeared to be associated with *B. lathyri* in the host plant but there was no evidence that these white organisms aided infection in any way.

Following the successful inoculation experiments detailed above came a period throughout the entire summer of 1921 during which numerous abortive attempts to produce disease of beans growing in the open by inoculation with the strains 33 and 45 c caused doubt as to whether the causal organism had really been obtained. All these doubts, however, were dispelled in September when the same strains which had been used all through the summer gave very positive evidence of infection. The weather conditions at this time led to very heavy dews in the early mornings followed by hot days, conditions which seem to favour the development of the disease. The previous failures to produce disease are doubtless accounted for by the extreme drought of the summer months, the plants probably rarely—if ever—attained full turgidity and hence enjoyed immunity against the bacterial attack.

A few examples of the September experiments may be given.

I.

8. ix. 21. *Leaf inoculation.* A drop of fluid culture was placed on the leaf and the surface gently pricked with a glass needle. Controls similarly inoculated with boiled culture.

Twelve pricks were made with each of three strains of *B. lathyri.*

13. ix. 21. Twenty-one chocolate spots well developed; lesions extend right through thickness of leaf; most of the remaining points show a slight reddened area round the wound; a few are negative, these being situated at the top of leaf where they would tend to dry up more quickly than at the base. Controls all negative.

II.

23. ix. 21. *Leaf inoculations* with organisms isolated from the previous experiment. One hundred and ten inoculated spots and 130 control spots with boiled culture.

28. ix. 21. Eighty-three large chocolate spots about $\frac{1}{2}$ cm. diameter. Control pricks all negative.

3. x. 21. No further development. Two leaves photographed (see Pl. VIII, fig. 2).

III.

8. ix. 21. *Stem inoculations.* Six plants grown outdoors inoculated with the same strains of *B. lathyri* as were used in Exp. I. Inoculation by a hypodermic needle in the axil between the stem and a leaf. Six control plants similarly inoculated with boiled culture.

13. ix. 21. *Controls.* Point of inoculation healed, no reddened area. Pith browned where the needle had entered.

Inoculated plants. Four plants show a reddish-brown lesion about ½–1 cm. diameter round the point of infection with a few streaks running up the stem to a height of 4 cms. Otherwise the exterior of the stem is unmarked. The pith is browned for a distance of 15–18 cms. up the stem, no lesions in the cortex. The other two plants show the same exterior lesions on the stem and local browning of the pith but no spread up the pith.

IV.

15. ix. 21. *Leaf inoculation* with Strain 70. One hundred spots, all negative. The authors would draw special attention to the failure of this strain to produce disease since careful comparison of Strain 70 with other strains of *B. lathyri* proved it to be in other respects identical. Detailed description of these comparative experiments are to be found in the communication which follows (see p. 204 of this Number).

Inoculation of Broad Bean Cotyledons.

The seed was sterilised externally by means of mercuric chloride and the testa slit open with a sterile knife, the cotyledons were placed in moist chambers and inoculated at the centre with a drop of a suspension of *B. lathyri*, the surface being gently scratched with a sterile needle. Controls were prepared in exactly the same way with boiled suspensions of the organism.

Experiments were made with ten different strains of *B. lathyri* and in every case resulted in browning and destruction of the tissue to a depth varying with the virulence of the strain from 1 mm. to the entire thickness of the cotyledon; the controls were practically unaffected (see Pl. VIII, fig. 3). Where the virulence of the strain was comparatively weak the diseased area was surrounded by a darker brown zone of cork.

Similar attempts at inoculation were made with the other organisms, *e.g.* strains 10 and 20 of *P. Phaseoli* and various white organisms mentioned above. All these attempts failed to produce any symptoms of disease.

Evidence of rapid desiccation of B. lathyri *in lesions.*

The fact that attempts to isolate the causal organism from lesions in the pith and from many spots on the leaves proved abortive suggested that the organism possessed small power of resistance to dry conditions. Bean plants growing in the open were sprayed with a suspension of *B. lathyri* on 28. vi. 21. Typical red brown spots developed on the under surface of the leaves. On 12. vii. 21 attempts to isolate the organism from these spots failed entirely.

In a second experiment on 8. vii. 21 leaves were infected by prick inoculation. On 15. vii. 21 six spots cut out with sterile instruments but without surface sterilisation were dropped into broth tubes. After 24

hours' incubation a loopful of the broth was plated. Four of the tubes gave *B. lathyri* and two failed to do so. On 22. vii. 21 six spots were again cut out and treated as before, with the results that two gave pure cultures of *B. lathyri* and four failed. In a third experiment on 8. ix. 21, 20 days after inoculation plating from one spot gave no growth of *B. lathyri* and from a second spot only 12 colonies of the causal organism developed from the whole of the diseased tissue of the spot.

The restriction of the disease to small areas on the leaves and to narrowly circumscribed streak lesions on the stems is therefore fully explained by destruction of the organism by desiccation of the diseased tissue.

The Spread of the Disease.

As stated earlier the spread of this disease is mainly the result of certain favourable atmospheric conditions. Wind dispersal of the organism is undoubtedly a strong possibility; the organism thus carried falling upon the surface of the plant wetted by rain, dew or fog during warm weather has ample opportunity to multiply and produce stomatal infection.

Many of the diseased crops were smothered with aphides and these can undoubtedly carry the organism from plant to plant as has been shown by allowing an aphis to walk across the surface of an agar plate. After removal of the aphis and incubation of the plate many yellow colonies developed and these were proved to be those of *B. lathyri*.

Aerial infection, though very prevalent, is not the only means of spread of disease. It is perfectly obvious to the observer that infection of the plant must often arise either from the soil or the seed. Field notes made at Carmarthen and elsewhere established the fact that the disease often starts from the base of the plant and works upwards. On one small garden plot which held about 200 plants of broad bean a *single* individual was typically diseased, smothered with brown spots and stem lesions from top to bottom. A photograph of this plant appears in Pl. IX, fig. 4. The neighbouring plants were normal, so obviously this must have been a case of seed infection, or an isolated case of wound infection below the soil level, which is less probable.

Bruchus rufomanus *as a cause of seed infection.*

Some bean seed from a badly diseased crop of the previous year was obtained in March, 1921, and many of the beans were found to be bored by the bean beetle, *Bruchus rufomanus*. These were separated from

sound beans and soaked overnight in water, the water containing bored beans became quite turbid while that containing sound ones remained almost clear. The liquids were plated out and from the turbid water an almost pure plate of yellow organisms resulted. These had all the appearance of colonies of *B. lathyri*. The experiment was repeated with seed from one of the well-known seedsmen, a sample which contained a large number of beetle-bored seed. The seed was poured straight from the bag into a sterile dish from which bored and sound beans were selected and placed in sterile vessels containing 50 c.c. of sterile water as under:

Beaker A. 12 sound beans.
B. 12 beetle-bored beans.
C. 8 sound plus 4 beetle-bored beans killed by boiling.
D. 12 beetle-bored beans boiled for five minutes.

After 24 hours at room temperature a loopful of water from each was plated on agar with the following result:

Beaker A. 32 colonies developed all yellow and proved to be *B. lathyri*.
B. 760 yellow colonies, *B. lathyri*.
460 white colonies, *B. subtilis* type.
C. 34 yellow colonies, one white.
D. 2 colonies of penicillium only.

This experiment was repeated with beans from another source.

Ten sound beans gave one white colony.

Ten beetle-bored beans gave 16 white colonies; two Penicillium colonies.

No colonies of *B. lathyri* developed from these beans, but, as the following table shows, streak disease developed to a certain extent when the bored beans were planted.

The table below shows the result of planting beans from these two sources designated *A* and *B*.

No. of beans planted	Source	Condition of beans	No. germinated	% germination	Condition of plants	% of diseased plants
50	A	Sound	44	88	35 healthy 9 slightly streaked	20
50	A	Beetle-bored	6	12	1 healthy 5 streaked	83
15	B	Sound	15	100	All healthy	0
15	B	Beetle-bored	14	93	12 healthy 2 diseased	14
10	B	Spotted with chocolate spots (see Pl. X, fig. 5)	10	100	8 healthy 1 slightly diseased 1 badly „	20

The "spotted" beans referred to above had several chocolate spots on the testa, these extended through the testa but no lesions were observed on the cotyledons beneath. The plant arising from one of these seeds and said to be "badly diseased" was interesting in its development; the first shoot came up only one inch above the ground, had a wide chocolate coloured streak up one side and several smaller lesions, growth stopped and the shoot died. A second shoot was put out and grew to a height of four inches, it also showed disease in a broad streak 2–3 mm. wide and 3 cm. long starting at the junction with the cotyledon. This shoot died back and a third one developed, grew to a height of nine inches and appeared to be perfectly healthy when the plant was dug up for examination. The tap root was unmarked but four of the secondaries were streaked and the cotyledons were spotted throughout with chocolate-coloured lesions (Pl. X, fig. 6). *B. lathyri* was isolated from the cotyledons and from the second shoot. The entire third shoot was ground with sand and water and plated but no *B. lathyri* developed, it therefore appears that this shoot had outgrown the disease. Reference may be made here to a means of control of tomato "stripe" caused by the same organism where by the cutting off of the diseased stems laterals often give rise to healthy plants.

Methods of Control.

The "streak" disease of beans is one which is always present amongst field beans but usually to such a small extent that it need not be contemplated seriously. The disease assumes an epidemic form only under exceptional climatic conditions; these exceptional conditions prevailed in 1920 and gave rise to a bad epidemic. A repetition of the trouble was expected in 1921 as the 1920 seed was badly infected; inspection of the crops showed, however, only an insignificant amount of the disease in this extremely dry year.

Where disease has occurred seriously it would be advisable, before another crop of beans is carried, to dress the land well with potash, since it has been shown that this treatment has successfully checked the ravages of the bacillus upon tomato plants, and there is every reason to expect that it would be equally effective in controlling the disease of beans. Further, it would be well to examine the seed carefully and to reject any showing an excessive amount of boring by beetles, and to sterilise the seed by soaking in weak lysol or formaline.

When the disease appears in the crop and there is at the same time an infestation of the black fly, spraying to destroy these would almost

Fig. 1.

Fig. 3.

Fig. 2.

PAINE & LACEY.—Studies in Bacteriosis. IX. "Streak Disease of Broad Beans." (pp. 194—203.)

[From the *Journal of the Ministry of Agriculture* by permission of the Ministry of Agriculture and His Majesty's Stationery Office]

Fig. 4.

PAINE & LACEY.—Studies in Bacteriosis. IX, "Streak Disease of Broad Beans." (pp. 194 -203.)

Figs. 5 and 6.

PAINE & LACEY.—Studies in Bacteriosis. IX. "Streak Disease of Broad Beans." (pp. 194—203.)

certainly save the crop to a considerable extent. While in the past spraying for black fly has not commended itself to the grower, recent improvements in the manufacture of insecticides have made possible the destruction of this pest.

In conclusion the authors wish to express their thanks to Messrs A. D. Cotton, S. P. Wiltshire and W. H. Broadbent of the Ministry of Agriculture for notes on the disease as it occurred in the field and to Mr J. C. F. Fryer for notes on the habits of the bean beetles.

SUMMARY.

The "streak" disease of beans (Chocolate Spot Disease) is caused by *B. lathyri*.

Moist and warm conditions are responsible for the appearance of the disease in epidemic form.

The disease is spread by wind, black fly, and the bean beetle.

Methods of control are suggested.

REFERENCES.

(1) MANNS, T. F. and TAUBENHAUS, J. J. (1915). *Gard. Chron.* 53, p. 215.
(2) MANNS, T. F. (1915). Delaware College Agr. Exp. Sta. *Bull.* 108.
(3) PAINE, S. G. and BEWLEY, W. F. (1923). *Annals of Applied Biology*, x, 89.
(4) —— (1919). *Ibid.* VI, 183.
(5) PAINE, S. G. and LACEY, M. S. (1923). *Annals of Applied Biology.* x, 204.

EXPLANATION OF PLATES VIII—X

PLATE VIII

Fig. 1. Showing the characteristic chocolate-coloured spots on the leaf, the large patches on the margins are due to secondary infection by *Botrytis*.

Fig. 2. A. Showing spots as the result of prick inoculation of bean leaves with *Bacillus lathyri*. B. Showing needle prick marks in control leaves.

Fig. 3. Showing result of inoculation of bean cotyledons with *B. lathyri*; inoculated half of bean on the right, control half on the left.

PLATE IX

Fig. 4. Showing the characteristic streak lesions on the stems and chocolate-coloured spots on the leaves.

PLATE X

Fig. 5. "Spotted" beans referred to in the table on p. 291.

Fig. 6. Plant raised from a spotted bean. A. First shoot (diseased). B. Second shoot (diseased). C. Third shoot (healthy).

(*Received July 25th*, 1922.)

STUDIES IN BACTERIOSIS. X

"THE USE OF SERUM-AGGLUTINATION IN THE DIAGNOSIS OF PLANT PARASITES"

By SYDNEY G. PAINE and MARGARET S. LACEY.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

INTRODUCTION.

During the course of investigations of the "Stripe" disease of tomato (5) and of the "Streak" disease of beans (6), both of which are caused by the same parasite, *Bacillus lathyri*, certain other yellow bacteria were frequently isolated at the same time as this organism. Chief amongst these was one which bore a certain superficial likeness to *B. lathyri* but differed from it in its physiological behaviour; in fact it gave all the characters of *Pseudomonas Phaseoli*. These two organisms possess many distinguishing features, *e.g.* in their sugar fermenting properties and in their power of liquefying gelatine. However, Harding and Morse (2) showed that various strains of *Bacillus carotovorus* might differ widely in fermentation reactions, but on simultaneous cultivation under identical conditions such differences disappeared, and the sugar fermentations could be relied upon for diagnostic purposes if certain previous cultural precautions were taken. Later, Morse (4) showed that the power of liquefaction of gelatine was also liable to change under similar treatment. With this in mind it seemed possible that the organism which appeared to be *Pseudomonas Phaseoli* might, in reality, be a degenerate form of the species *B. lathyri*. Several strains of both organisms were therefore "trained" simultaneously in bouillon by daily transfers until the tenth day when they were plated on bouillon agar, and subsequently inoculated into the various sugar and other media generally used for diagnosis. The results given in Table II (p. 207) showed that even after such treatment the organisms retained their individual characters.

In order further to verify these results it seemed advisable to have recourse to animal inoculation in the hope that specific agglutinins would be produced. This is not the first instance of the use of serological tests in connection with plant organisms. Zipfel (8) in 1912 and Klimmer and Krüger (3) in 1914 were able to group various strains of *Bacillus radicicola*

according to their serum agglutination phenomena and Doidge(1) in 1917 found that the serum of a rabbit inoculated with *Bacillus citrimaculans* agglutinated that organism in dilutions down to 1–2000.

EXPERIMENTAL.

The serum used in this work was prepared by Dr St John Brooks of the Lister Institute of Preventive Medicine to whom, and to his assistant, Miss M. Rhodes, the authors desire to express their sincere thanks.

Three organisms were employed for inoculations:

(1) Strain 33, *Bacillus lathyri* from Bean.

(2) Strain 20, *Pseudomonas Phaseoli* from Sweet Pea.

(3) Strain 40, *Aplanobacter Michiganense* E.F.S. from Tomato.

1 c.c. of a thick emulsion of the several organisms in normal saline was injected intravenously into young rabbits. No pathogenic symptoms were observed in any case so the dose was repeated a week later. At the end of the second week a sample of serum from each rabbit was collected and tested for agglutinins; strain 33 agglutinated with its serum diluted 1 in 2000, strain 20 with its serum diluted 1 in 200, but strain 40 failed to show agglutination. A third dose was given to rabbits "20" and "40" a week later, and a fourth dose to rabbit "40" after the lapse of another week. Serum was collected from rabbit "33" four weeks, and from rabbits "20" and "40" five weeks after the first inoculation. At this time the titre of 33 and 20 was 1 in 2000 in each case, but 40 again failed to agglutinate in any dilution.

Method of agglutination. Dilutions of the serum were made with water to 1–10, 1–50, 1–100, etc. 0·5 c.c. of an emulsion in water from a 24-hours' culture on agar was placed in the tubes and 0·5 c.c. of the diluted serum added, thus giving dilutions 1–20, 1–100, 1–200, etc. In the control tubes 0·5 c.c. of water took the place of the serum. The tubes were immersed in water at 30° C. and incubated for four hours. Observations were then made and verified the next morning after standing in the cold overnight.

While all strains of *B. lathyri* exhibit marked agglutination with the 33 serum, the agglutinating power of the serum is noticeably different for different strains. This is not attributable to the source from which the strains were derived. Numbers 2 and 3, for instance, were isolated from the same sweet pea plant, they were kept as separate strains on account of a slight difference in the colour developed in the growth on agar; they gave identical fermentation reactions, yet they show marked difference in agglutination.

Table I. *Showing agglutination of various strains of* Bacillus lathyri.

++ indicates strong and + slight agglutination.

No. of strain	Host plant	Serum dilutions 1–500	1–800	1–1000	1–2000	1–2500
1	Broad bean	++	++	+	—	—
2	Sweet pea	++	++	++	+	—
3	„	++	++	—	—	—
4	Inoculated broad bean	++	++	—	—	—
5	„ „	++	++	—	—	—
6	Broad bean	++	++	++	+	—
7	„	++	—	—	—	—
8	Clover	++	+	—	—	—
21	Washings of beetle-bored beans	++	++	++	+	—
26	Washings of uninjured beans, from the same packet	++	—	—	—	—
45	Broad bean	++	++	++	+	—
18	Sweet pea	++	+	—	—	—
33	Broad bean	++	++	++	++	+
Laboratory stock culture	Tomato	++	++	++	+	—
70	Broad bean	++	++	—	—	—

Again, *B. lathyri* from a tomato plant agglutinated at a dilution of 1 in 2000, while strain 7 from a broad bean, and thus from the same host as the strain used in preparation of the serum, agglutinated at a much higher point, namely, 1 in 500.

Moreover, the variation shown throughout the table cannot be attributed to the length of time the strains have been cultured on artificial media, for strains 2 and 3 were isolated at the same time and underwent exactly parallel treatment; this is also true of strains 21 and 26, while strains 1, 6 and 7 were isolated within a week or two of each other and show considerably different degrees of agglutination. No explanation of this variation is at present advanced. It is interesting to note, however, the occurrence of serological strains amongst plant pathogens which seem to be exactly similar to those which occur amongst the human pathogens. Some very interesting results with regard to serological strains in the enteric and dysenteric groups of bacteria have recently appeared in a preliminary communication by Walker(7). Agglutinable and dys-agglutinable forms may be obtained at will from one and the same strain of a bacillus and both may revert or may be converted mutually the one into the other.

In Table II are given the results of comparative experiments with various strains of *Bacillus lathyri*, *Pseudomonas Phaseoli* and *Aplanobacter dissimulans* and *michiganense*.

Table II.

	Strain	Lowest dilution at which agglutination takes place		Sugar fermentation with production of acid in			Nitrate reduction	Diastatic action	Liquefaction of gelatine	Motility
		Serum 33	Serum 20	Glucose	Lactose	Sucrose				
33	*B. lathyri* from broad bean	1–2500	1–20	+	—	+	+	+	Very slow	Motile by peritrichous flagella
Stock culture	*B. lathyri* from tomato	1–2000	—	+	—	+	+	+	„	„
2	*B. lathyri* from sweet pea	1–2000	1–20	+	—	+	+	+	„	„
26	*B. lathyri* from winter bean	1–500	1–20	+	—	+	+	+	„	„
5	*B. lathyri* from broad bean	1–800	1–20	+	—	+	+	+	„	„
70	„ „	1–800	1–20	+	—	+	+	+	„	„
94	*B. lathyri* (?)	1–100	1–100	—	—	—	—		None	—
20	*P. Phaseoli* from sweet pea	—	1–2000	Slight after 5 days	—	—	—	+	Rapid	Motile by single polar flagellum
*b**	*P. Phaseoli* from broad bean	1–20	1–20	Slight after 5 days	—	Slight after 5 days	—	+	Not so rapid as 20 but not so slow as *B lathyri*	„
10	*P. Phaseoli* from Phaseolus leaf	—	1–100	—	—	—	—	+	Rapid	„
9	*A. dissimulans* from Phaseolus leaf	—	—	Very slight after 5 days	—	Very slight after 5 days	—	+	„	Not motile
Stock culture	*A. dissimulans* from tomato	—	—	Very slight after 6 days	Very slight after 9 days	Very slight after 6 days	—	+	„	„
40	*A. michiganense* from tomato	—	—	Slight after 13 days	Slight after 13 days	Slight after 13 days	—	+	Very slow	„

* Control with normal rabbit serum.

In the above table it will be seen that *Bacillus lathyri* and the two organisms, *Pseudomonas Phaseoli* and *Aplanobacter dissimulans*, which often appear on isolation plates from tissues of plants diseased by *Bacillus lathyri*, retain their distinguishing morphological and physiological characters even after the preliminary training mentioned above (p. 204). They must therefore be regarded as distinct species. Strains 94 and *b*, however, seem to be intermediate types between *Bacillus lathyri* and *Pseudomonas Phaseoli*, this is shown in their physiological reactions and in the fact that they agglutinate equally with agglutinating sera of both species. *Bacillus lathyri* in all but one of the strains was agglutinated slightly but definitely with the serum of *Pseudomonas Phaseoli*. This seems to show a relationship similar to that existing between *Bacillus typhosus* and *Bacillus paratyphosus A* and *B* and suggests the possibility that one of the species may have arisen by mutation from the other. If such mutations occur in the tissues of the host plant an explanation would be found for a phenomenon with which the plant pathologist is very familiar, namely, that on plating from diseased tissues colonies develop which appear to be all of the same organism, yet few of these, and sometimes quite a small percentage, possess any virulence for the host plant; it is possible that the non-virulent forms are mutants of the virulent one.

The *Aplanobacters* seem to be entirely distinct from *Bacillus lathyri* showing quite different physiological behaviour and no agglutination with either sera 33 or 20 and, as stated above, even with the serum 40 prepared from *Aplanobacter michiganense*.

SUMMARY.

(1) Serum agglutination has been used in order to determine possible relationship with *Bacillus lathyri* of certain yellow organisms which occur in the tissues of plants infected by that organism.

(2) One of these gave all the reactions of *Pseudomonas Phaseoli*.

(3) *Pseudomonas Phaseoli* and *Bacillus lathyri* gave distinct cultural characters, and these were retained even after the two organisms had been "trained" by ten transfers through the same medium.

(4) The two organisms seem to be susceptible to group agglutination, *Bacillus lathyri* being agglutinated with the serum of *Pseudomonas Phaseoli*.

(5) Intermediate forms between these two organisms are found which agglutinate equally with the sera of both species.

(6) The suggestion is made that one species may have arisen in the tissues of the plant as a mutant from the other.

(7) *Aplanobacter dissimulans*, another organism often associated with *Bacillus lathyri*, appears to bear no sort of relationship with the latter.

(8) *Aplanobacter michiganense* failed to produce an agglutinating serum on inoculation into a rabbit.

REFERENCES.

(1) DOIDGE (1917). *Annals of Applied Biology*, III, 53.
(2) HARDING and MORSE (1909). *New York Agric. Exp. Sta., Tech. Bull.* No. 11.
(3) KLIMMER and KRÜGER (1914). *Centralbl. f. Bakt.* 2 Abth. XL, 256.
(4) MORSE (1917). *Jan. Agric. Research*, VIII, 79.
(5) PAINE and BEWLEY (1919). *Annals of Applied Biology*, VI, 183.
(6) PAINE and LACEY (1923). *Ibid.* X, p. 194.
(7) WALKER (1922). *Proc. Roy. Soc. B.* 93, p. 54.
(8) ZIPFEL (1912). *Centralbl. f. Bakt.* 2 Abth. XXXII, 97.

(*Received July 25th*, 1922.)

ON THE RELATIVE IMPORTANCE OF CERTAIN COMMON GRASSES AS HOST PLANTS OF *OSCINELLA FRIT*, LINN.

By NORMAN CUNLIFFE, M.A. (Cantab. et Oxon.),

Christopher Welch Lecturer in Economic Zoology, University of Oxford.

Previous experiments(1) have indicated that under artificial conditions the frit fly exhibits a certain degree of preference for *Arrhenatherum* species among grasses. In order to determine what occurred under natural conditions (for the fly) in the field, the seed of 12 different species of grasses, known to be liable to infection, was sown in duplicate on plots of one square yard, the plots being situated ten yards from an oat crop. Sowing was deferred until 20. iv. 22 to ensure infestation; the unfavourable conditions for germination prevailing at this time and also the varying quality of the seed greatly affected the numbers of plants obtained. The plants were collected on 11. vi. 22, being sampled at hazard where numerous, and were examined in the laboratory for signs of frit attack. Flies were reared from the puparia[1] obtained during this scrutiny and as no species other than *O. frit* emerged, it seems legitimate to assume that all the larvae also were those of *O. frit*.

	Series I				Series II				
Host plant*	No. of plants	No. of puparia	No. of larvae	Infestation per 100 plants	No. of plants	No. of puparia	No. of larvae	Infestation per 100 plants	Infestation mean value
Arrhenatherum avenaceum	66	16	36	78·8	56	22	26	85·8	82·3
A. avenaceum var. *bulbosum*	60	22	24	76·7	50	12	30	84·0	80·3
Hordeum murinum	42	10	32	100·0	40	8	14	55·0	72·5
Agrestis myosuroides	40	6	24	75·0	40	11	16	67·5	71·2
Lolium italicum	88	10	24	37·6	54	10	26	66·7	52·1
L. perenne	51	2	2	7·8	70	—	4	5·7	6·7
Festuca pratensis	12	—	1	8·3	10	—	—	—	4·1
Dactylus glomerata	36	—	2	5·6	21	—	—	—	2·8
Holcus lanatus	50	—	—	—	43	—	—	—	—
Bromus sterilis	47	—	—	—	37	—	—	—	—
Oat crop (adjacent—sown, March 17th)	49	15	18	67·3	—	—	—	—	67·3

* *Hordeum pratense* and *Avena flavescens* failed to grow.

[1] Only 34·5 per cent. of the larvae had pupated by 11 vi. 22.

The data obtained from these plots are collected in the preceding table. The column showing the number of puparia discovered includes the stems obviously drilled by the larvae but not containing either stage at the time of examination. These were very few in number, but may be the cause of slight inaccuracy since each such stem may have contained two or more larvae. As the influence of the grass hosts on the field population seems likely to become an important factor, the comparison between the grass species has been based on the infestation per hundred plants.

These figures are comparative only in the sense that they refer to plants produced from seed germinating about the same time[1]. There

[1] Although it is notorious that the liability of oat plants to infestation is in some degree correlated with growth, the factors determining differences in susceptibility to attack have not yet been investigated. In 1922 an experiment, as yet unconfirmed, conducted to ascertain the relation between age of plant and intensity of attack yielded a somewhat unexpected result and is of interest in this connection.

Oat seed was sown in 9″ pots, every seven days from April 6th to May 4th inclusive, the resulting plants being protected from the fly in a cold greenhouse. On May 10th, five pots of each series were distributed regularly under a large muslin outdoor cage and on May 16th the cage was infected with 200 flies from the field, these flies being allowed to remain in association with the plants for a period of fifteen days. On May 10th the total numbers of plants in the five series were in order of age, (*a*) 117, (*b*) 87, (*c*) 85, (*d*) 73, (*e*) 68, while the average growth in centimetres was (*a*) 19, (*b*) 15, (*c*) 11, (*d*) 6, (*e*) 0·5.

Examination on July 18th for evidence of larval working, showed that the distribution of the attack, as denoted by the number of bored stems in each pot in each series, was (*a*) 0, 1, 0, 3, 0, (*b*) 0, 1, 3, 1, 1, (*c*) 3, 4, 4, 1, 1, (*d*) 1, 0, 0, 1, 8, (*e*) 0, 0, 1, 0, 0, *i.e.* the percentage infestation in order of age was 3·4, 6·9, 15·3, 13·7 and 1·5 respectively. The comparatively high value obtained in the fourth series is dependent on an abnormal infestation in one pot and should probably be of the order of that of the second series. It is possible that either morphologically or physiologically the plants in this one pot belonged to the third series.

The interest of these data lies in the fact that intensity of infestation was not inversely related to age, there being a period, early in the cycle certainly, when the main stem was in the most susceptible condition. Now the plants appeared above the surface of the soil on the average in seven days from the date of sowing, thus the ages of the plants in each series at the time of infection (May 16th) were (*a*) 33, (*b*) 26, (*c*) 19, (*d*) 12 and (*e*) 5 days respectively. The third series being most severely attacked it would appear that the most susceptible period in the early part of the plant cycle occurred about the 19th to the 26th day from the date of appearance above soil, *i.e.* while the plants were growing in height from 15 to 25 cm. During the infection period the first series had tillered but the tillers were only approaching susceptibility as the infestation was slight; the second series had practically passed out of the most susceptible stage; except for the abnormally infested pot, the fourth series was not heavily infested, although the susceptible stage fell entirely within the infection period—this stage was reached towards the end of this period, when the fly population was decreasing rapidly; the fifth series barely reached the requisite age and suffered very slightly.

No explanation of this variation in susceptibility can be advanced at present, as the morphological and physiological changes taking place in the oat plant during growth are apparently unknown.

seems to be no information readily available as to when germination of the seed of wild grasses occurs in nature.

Under these conditions, the *Arrhenatherum* species were again most heavily infested, although *Hordeum murinum* and *Agrestis myosuroides* followed closely. The difference between the two *Lolium* species is very striking, *L. italicum* being attacked fairly severely while *L. perenne* practically escaped. It is also evident that the fly had a decided preference for certain of these grasses rather than for oats under these particular conditions. The oat plants were in great preponderance numerically but they were more advanced in growth than the grasses, therefore it would appear from these data either that the fly possesses the faculty of selecting suitable plants for oviposition or that, if oviposition is indiscriminate, the larva in some cases fails to reach maturity for reasons connected with plant morphology or physiology. In a previous publication (2) it has been recorded that *Alopecurus myosuroides*, growing wild in the fields among oat plants, may be the more heavily infested, this observation confirming the present data[1]. Records of this nature indicate that grasses, either wild or cultivated, may be of considerable importance in relation to the prevalence of the fly in the field. If the field population is dependent on the grasses rather than the oats, the problem of reducing the extent of the grain infestation is rendered more complex.

REFERENCES.

(1) Cunliffe, N. (1922). *Ann. App. Biol.* ix, No. 2, 165–168.
(2) —— (1921). *Ibid.* viii, Nos. 3 and 4, 170–181.

[1] If all graminaceous host plants have susceptible periods like the oat, different results may be expected from other age associations of these grasses, because the position of the susceptible period will not necessarily be the same in the cycles of different species.

(*Received March 28th*, 1923.)

FURTHER CONTRIBUTIONS TO THE BIOLOGY OF FRESH WATER FISH. EFFECT OF VARIOUS TRADE WASTES

By W. RUSHTON, A.R.C.S., D.I.C. (Lond.), M.Sc. (Ill.), F.L.S.

In the April number of the *Annals of Applied Biology*, IX, 1, attention was drawn to some of the effects of trade effluents on the life of the spermatozoa of trout. Further observations have been carried out on young trout using a series of effluents of varied kinds. A certain amount of work has been done on somewhat similar lines in England, the most recent being that of the Joint Board of the Ministry of Transport and Fisheries with regard to the toxic effects of road tar, the Ministries up to the present having issued an interim report but not the full report.

At the commencement of these experiments a series of tests were carried out using the various inorganic and organic substances which are the basis of many trade effluents.

The effect on young trout of most of the alkalies and some salts in dilute solution is to cause a large exudation of mucous from the gills, fins and skin, some being more severe than others, *e.g.* sodium hydrate and sodium carbonate readily cause much mucous to appear at once in stringy masses until such times as the gill covers cannot move on this account. Microscopic examination of the epithelia covering the gills after death show them to be swollen and cleared of granules. Dilute acids, on the other hand, coagulate the mucous as a rule and the epithelial cells are usually shrunk and charged with granules. The effects of distillery effluent were dealt with in the April number, the effects of Flaxwater, Tannery Waste, Woollen Mill, Paper Mill and a gas works are dealt with here.

The effluents vary in effect as would be expected as distillery effluent heavily charged with CO_2 makes the fish lethargic from which condition they recover if removed to fresh water in time. Others have a distinct toxic effect which is fatal almost at once, whilst others bring about a slow lingering death.

FLAX WATER.

Water in which flax has been retted is known to be poisonous to fish. In Ireland damage is done by its careless release from retting pits. Both green and dried flax are retted, a sample of dried flax was used for these experiments. Soft water is stated to be best for the purpose, but London tap-water was used in this case. The flax water was obtained by taking commercial dried flax and steeping it in water at laboratory temperature, the amount used was approximately 300 gms. of flax placed in 100 litres of water. After 24 to 48 hours a vigorous fermentation was set up, much gas rising to the surface. After three days gas production ceased and the liquid became slightly acid, which increased sufficiently to check the further action of the bacteria causing the fermentation. After a week onwards a gelatinous scum began to appear on the surface to a thickness of about an inch, and a strong sulphuretted hydrogen smell prevailed.

Fish were introduced at various stages of the process with the following results:

1. Introduction of yearling trout at the beginning of experiment. No marked effect was shown till after the expiration of 24 hours, when the fish showed signs of becoming sleepy with a tendency to remain in one place refusing to take food, and at the end of two days all the fish originally introduced were dead.

2. Fresh fish introduced at this stage lived for 4–8 hours appearing from the first very lethargic.

3. Two rainbow trout were introduced into the flax water after four days when the fermentation was beginning to subside and the water had become dark brown in colour. The fish were at first very active and made determined efforts to jump out of the tanks, then gradually settled to the bottom breathing rapidly and slowly turned on their backs, with gill covers widely extended. Occasional darting took place and death occurred after about 45 minutes. When dead, the fish had their mouths and gill covers wide open with the gill filaments congested with blood.

4. A quantity of an eight-days' old culture was diluted with an equal volume of water, and, into it introduced two rainbow trout. The effect was similar to the effects with the younger culture but more rapid. One of the fish died within ten minutes, the remaining one was at once removed to fresh running water and recovered from the effects in half an hour. The gills of the dead one were intensely red at the tips of the filaments.

5. The culture was allowed to continue for over two months, during the latter part of which the smell of sulphuretted hydrogen was very apparent. Equal parts of an eight weeks' culture and water were taken, into which were introduced four rainbow trout and one stickleback. As soon as introduced, the fish began darting from side to side keeping near the surface, the stickleback being least affected. All the fish turned on their backs in under five minutes with much aimless darting, and within ten minutes one of the trout was dead. Removing the other three and placing one in running water, another in diluted flax water with a current of water running through, and the third into flax water into which powdered slaked lime had been well mixed and allowed to settle, gradual recovery took place, the last to recover being that where lime had been used.

From the above experiments it would appear that there are three causes which render flax water harmful to fish:

1. Excess of carbon dioxide and a deficiency of oxygen.
2. A rise in acidity.
3. Presence of sulphuretted hydrogen in the water.

Enquiry has shown that damage to fish life in streams occurs when the flax water is liberated at low water and that in times of flood little or no harm appears to follow.

TANNERY WASTES.

The wastes from tanneries are known to be harmful to fish and pollution by them is not uncommon. A few months ago I was allowed to visit a tannery in Scotland where pig skins are prepared for usage in the preparation of hand-bags, purses, etc. and directed to take samples of the four main effluents previous to discharge into the drains.

The effluents were four in number:

1. Lime solution which is used to swell the hide and loosen the hair for easy removal. This effluent contained much lime in suspension and large amounts of organic matter, and gave off a very offensive smell.

2. Bate liquor. The composition of this in the fresh state I was unable to ascertain, but it is used to steep the hides after the lime treatment and neutralises the action of the lime, bringing the hides into a pliable condition. The hides if kept too long in this liquor will putrefy and disintegrate. When cold, it is neutral to phenol phthalein and litmus; on warming, it becomes pink and gives a slight alkaline reaction.

3. Tan liquor is used after bate liquor. This is obtained by steeping bark and nut shells in water. When most of its properties are spent, the

remains are run to waste which at this time gives a strong acid reaction and is heavily charged with foreign matter of inorganic and organic origin.

4. Chrome liquor. Used after the tan liquor. This contains potassium bichromate and sodium hyposulphite in varying amounts. As the various effluents are mixed before turned to waste, varying results would arise as one liquor had a tendency to counteract the other.

The following experiments were done using separate wastes and mixtures, using yearling brown trout in all cases.

Exp. 1. This sample, direct from the tannery, had much lime in suspension which was allowed to settle out. Two and a half litres of the top liquor were decanted off and made up to 100 litres with tap water, giving a dilution of 1-40. Yearling trout introduced, soon became torpid, remaining motionless in one corner of the vessel for 30 minutes, when they gradually lost equilibrium and shortly afterwards died. Repeating this experiment but removing the fish to fresh water on first signs of turning on their side, it was found impossible to get the fish to recover and within an hour they were dead.

Exp. 2. A dilution of 1–1000 was made and fish introduced which, after a period of two hours, appeared to be dead, but on transference to quick running water they recovered in about four hours.

Exp. 3. Bate liquor. A dilution of 1–40 of this liquor seemed to have little effect on the fish beyond a slight increase of respiration accompanied by some sluggishness. Leaving the fish periods of two, four, eight and ten hours and then transferring to fresh water, the fish soon appeared normal.

Exp. 4. Tan liquors. Tests were made with dilutions of 1–40 and 1–1000, and all symptoms were similar to what one gets if solutions of tanning from cutch are used. Cutch is a commercial substance of vegetable origin much used in treating fishing nets and sails. It consists of fragments of a hard, dark-coloured resinous substance and contains about 20 per cent. of tannin.

Two gms. of cutch were extracted with water and made up to 100 litres, the result is a dark brown liquid resembling weak tea. Fish introduced into this, soon show signs of disturbance. The head moves quickly from side to side in quick jerks, they swim wildly and irregularly from side to side for about 15 minutes then settle down in one corner, and for four hours never alter their position unless mechanically disturbed. After four hours the fish were removed to running water and after several hours gradually recovered their normal movements. For three days

afterwards whether tan liquor effluent or extract of cutch was used, the fish refused all food. The tannin liquor appeared to be partly swallowed, which would affect the mucous membrane of the oesophagus and stomach in a way which would seriously affect digestion, in addition to having a detrimental effect on the gills. Too long immersion of fish in this liquor proved fatal.

Exp. 5. Chrome liquor. This was a green-coloured effluent and a dilution of 1–4000 was made from the crude waste. Fish introduced were at first very active, moving restlessly about and keeping as near the surface as possible. After 45 minutes the fish had a tendency to swim in a vertical position head upwards with increased rate of respiration. Bubbles of gas occasionally escaped from the mouth. The fish were left for two hours in this solution after which they were removed to fresh water and for a time appeared normal, but within 24 hours the fish appeared out of sorts and died within four days. After death the mouths were closed, gills brownish-pink in colour and the epithelia covering the gills disintegrated and easily stripped off. Dissection showed the lining of the gut inflamed.

Exp. 6. A mixture of the four liquors, 100 c.c. of each being taken and made up to 100 litres. The mixture gave an alkaline reaction and contained a little suspended matter, brown in colour and gave off an offensive smell. Yearling trout placed in this mixture showed active disturbances at once and within 15 minutes were overcome beyond recovery. Repeating the experiment but removing to fresh water at the first signs of trouble, it was found impossible to recover the fish. This experiment was tried to test a theory prevalent in certain circles that while tannery effluents are harmful apart, a mixture of the four gives a harmless effluent.

EFFLUENT FROM WOOLLEN MILL.

Only diluted mixed samples of effluents from a woollen mill were available, and the constituents of these may vary from time to time due to the source of the waste products.

Three main sources of waste are possible:

1. From the wool scouring vats.
2. From the mordanting vats.
3. From the dye vats.

As a rule the effluent contains all three in different proportions. The method of wool scouring is well known and consists of washing the raw wool in a hot alkaline liquid which removes large quantities of natural

fats and dirt, when the process is finished the partly spent wool scouring liquor is usually poured into the neighbouring stream and consists of a dark soapy liquid which discolours the water and is strongly alkaline. The effect on fish is very marked and is very similar to what one gets if a strong freshly-made solution of sodium carbonate is used. The alkali stimulates the activities of the mucous cells around the gills and causes a heavy discharge of mucous which forms stringy masses at the edges of the fins and gill covers, finally causing the gills to be put out of action by its accumulation. Microscopic examination of the epithelia covering the gill filaments, shows the individual cells to be swollen up and cleared of granules which are the forerunners of mucous.

Fish introduced into the crude effluent at once show signs of irritation, the fish only being seen when near the surface. The eyes seem much affected, making unusual movements in a forward direction, the stringy mucous appears on the fins, etc. and although it takes many hours to kill the fish, it does ultimately result in death if they are left too long. If after two hours the fish are removed to running water, they recover in the course of a few hours.

It would appear that fish are able to withstand a sudden flush of wool scouring liquid more easily than many effluents, especially if the effluent is free from ammonia.

From mordanting vats. The mordants used at this particular woollen mill are alum and potassium dichromate, both of which alone are deadly to fish. The amount of mordant in the effluent is not usually very much, as most of it is recovered on account of its high price. It is usually coloured to varying extents with dye liquor.

Its effect on fish is similar to that of chrome liquor from a tannery. In no case was it possible to recover fish after an immersion of more than one hour if an amount of more than 1–40,000 of mordant was present.

EFFLUENT FROM A GAS WORKS.

The effluents from a gas works depend on the part of the plant from which they are drawn, but the particular effluent under consideration here was from the overflow of the ammonia collecting tanks which, as analysis shows, contained parts per 100,000:

Dissolved solids ...	68·00	Albuminoid ammonia	1·64
Suspended matter ...	35·00	Tar acids	23·84
Chlorine	21·30	Tar bases	6·00
Free ammonia ...	15·00	Dissolved oxygen ...	0·30

The sample was obtained from a river just where the effluent had entered and fish were killed by its action. The effect on trout

and grayling was marked by the gills being intensely red and swollen, the blood vessels of the gills gorged with blood and the covering epithelia swollen and free from granules. An excessive amount of mucous surrounded the gills. The effects were also shown in the gut. The oesophagus in all cases was locally inflamed and the walls of the stomach and intestine inflamed throughout. No parasites of any description were present in the gut. *Heart.* All vessels gorged with dark coloured blood.

The appearance throughout was similar to what one gets if dilute solutions of free ammonia are used on fish. The analysis shows 15·00 parts per 100,000 of free ammonia. Experiments using 3·00 parts per 100,000 have been found to kill trout in 20 minutes. Mason found 5–100,000 to kill in 47 minutes; Clark and Adams found 1–100,000 to kill in under an hour.

With tar acids the analysis shows 23·84 parts per 100,000. Experiments using 15–100,000 killed trout in 10 minutes; Rufter and Baker, using 33–100,000, killed trout in 5 minutes; Mason, using 1·5–100,000, killed trout in 15 minutes.

Gas water from top of the wells are similar to the sample under consideration. 1–100,000 killed perch in a few hours. Waste water from gas works, 0·5 per cent., kills trout, according to Knight, in 10–15 minutes. Analysis shows oxygen in solution to be under 50 per cent. normal, which is near the danger line for trout.

EFFLUENTS FROM PAPER MILLS.

The effluent from two paper mills has been investigated, one in Scotland, where the effluent enters a well-known salmon stream about 20 miles from the mouth, and another in the south of England on a tributary of the Thames. In regard to the northern one the effluent lodges in pools and has the effect of killing salmon which may find their way into it in times of low water, in this case as many as 25 fully-grown salmon being picked up within a few weeks.

Examination of one of these fish immediately after death showed the gill filaments very red and swollen, a slight excess of mucous in the mouth and on the tongue. Internally the liver was very dark on the outside and mottled in section. Spleen almost black, oesophagus, stomach and intestine intensely inflamed. Microscopic appearance of the gill epithelia showed the cells swollen.

A crude sample of the effluent was used on salmon fry, four of which were placed in a litre of effluent side by side with four in tap water, with

the result that of the ones subjected to the effluent, two died within two hours and the other two within eight hours. When dead, the mouth and gill covers were open to their widest extent, gill filaments greyish and swollen, covered with an excess of mucous, but not to the same extent as with a strong alkali. The blood vessels of the gut were much congested.

A second sample, more alkaline than the first, killed fry in three hours, strings of mucous appearing as a halo round the fins and body of the fish with the gill filaments intensely red and swollen.

In the case of the southern stream it has been possible to study the effects of the effluent on the stream itself, where its results can be seen. In this case from the mill are poured out two effluents, one from the washing machines and the other from a source which receives much of the waste oil. Both of these effluents are poured into a canal which runs somewhat parallel with the stream from which it overflows at various points into the stream.

The effect on the canal is that, below the effluent outflows, no fish of any kind exist and the vegetation is remarkably altered, many of the plants found above the mills being missing for some distance below. Examination of the various overflows from the canal to the river shows rich growths of fungi on the bed of the stream and the water is very cloudy. The effect on the stream is, that for some distance down, few, if any, trout are present, but above, they are plentiful and about four miles below they are plentiful. Although trout have been introduced from time to time in this polluted area they will not stay, but migrate up-stream or fall back down-stream.

A series of chemical analyses of the water from the river and canal above effluent outflow, crude effluents from the paper mill, diluted canal water flowing into main stream, and stream below effluent outflows give the following results which may vary from time to time depending on the rate at which effluent is entering canal.

The figures tend to show that the area affected by the effluent is sufficient to drive the fish away, and within the last few years has not only removed them but completely altered the nature of the stream in regard to vegetation and natural fauna, due to the constituents of the paper mill's effluents.

In many cases where chemical analysis has not yielded any result which would indicate the nature of the toxic substance, fish have been known to be affected, experience leads one to the conclusion that biological as well as chemical examination is important in investigating the deleterious effects of trade wastes on streams. The question of these

Analyses of Stream, Canal and Paper Mill Effluents.

	Stream above Paper Mill	Stream a mile below Paper Mill	Stream two miles below Paper Mill	Canal above Paper Mill	Canal 500 yds. below Paper Mill	Canal ¾ mile below Paper Mill	Canal two miles below Paper Mill	Crude eff. 1.	Crude eff. 2.
Physical characters	Clear	Slightly cloudy	Slightly cloudy	Clear	Cloudy	Cloudy	Cloudy	Opaque	Opaque
Colour in 2 ft. col.	Pale yellow	Cloudy	—	—	—	—	—	—	—
Reaction	Slightly alkaline	Neutral	Slightly alkaline	Slightly alkaline	Faintly acid	Faintly acid	Faintly acid	Acid	Acid
Analyses in parts per 100,000, *except oxygen in solution, which is in mg. per litre.*									
Solids in solution	20·00	22·00	15·00	22·00	40·00	30·00	25·00	120·00	85·00
Chlorine as chlorides ...	1·1	1·5	1·5	1·5	1·6	2·0	1·8	4·8	2·9
Ammonia free and saline ...	0·01	0·019	0·010	0·008	0·100	0·01	0·009	0·16	0·16
Ammonia albuminoid ...	0·014	0·026	0·020	0·012	0·86	0·02	0·018	0·22	0·20
Nitrates	0·2	0·25	0·10	0·20	0·3	0·18	0·150	0·54	0·38
Nitrites	nil	Trace	nil	nil	Trace	Trace	nil	Trace	Trace
Oxygen absorbed from potassium permanganate in 4 hrs. at 27° C.	0·03	0·09	0·082	0·03	0·10	0·52	0·45	2·00	1·6
Oxygen in solution, temp. 10° C.	9 mg.	8·7	7·5	8·5	8·2	4·5	4·5	1·0	2·0
Acidity as sulphuric acid ...	Slightly alkaline	Neutral	Neutral	Slightly alkaline	Faintly acid	Faintly acid	—	5·8	2·2

trade wastes is becoming of vast importance to the biologist, as so many of our streams are being gradually changed in character. They have also their economic bearing, indicating that many useful substances are being allowed to run to waste.

CONCLUSIONS.

1. Substances may be present in a stream in such small quantities as to escape chemical detection but may be deleterious to fish life.

2. The character of a stream may be totally altered, due to the effects of trade wastes being poured into it.

REFERENCES.

Stream Pollution Studies. State of New York Conservation Commission. 1922.
Interim Report for Pollution Commission. Ministry of Fisheries. 1922.

(*Received November* 30*th*, 1922.)

EFFECT OF WEIGHT OF SEED UPON THE RESULTING CROP

By WINIFRED E. BRENCHLEY, D.Sc., F.L.S.

(*Rothamsted Experimental Station.*)

(With 5 Text-figures.)

The question of the influence of the quality of the seed sown upon the resulting crop is one of paramount importance to the agriculturist, and has received a good deal of attention in the form of field trials. The results obtained are not very consistent, though the general trend is in favour of the well developed, heavier seeds. The reason for this apparent discrepancy may be due to the fact that the problem has really two aspects, which have not always been recognised and separated. In one aspect the relative weight of the seeds is estimated by the weight per bushel, in which case all the small, shrivelled and cracked seed that is screened out from a large bulk is included with the light seeds, giving a smaller weight per bushel than the remaining large seeds which are free from rubbish. In the other aspect the rubbish is not included, the comparison being made between good seed in each case, the difference being only in the size and weight of the individual seeds. This does not necessarily entail that the smaller seeds should give a less weight per bushel, though doubtless this does frequently happen. Then again the question arises as to whether the comparison is to be made with the crops obtained by sowing on similar areas

(1) equal quantities of seed by *weight*, the lighter seeds giving a larger number of plants per acre;

(2) equal quantities of seed by *volume*, in which case again the lighter seeds, being usually smaller, give more plants per acre than the heavier seeds;

(3) equal *numbers* of seeds, when the comparison becomes a true one as regards the productivity of individual plants.

All three methods have been used by investigators.

Zavitz(19) carried out experiments for several years with various field crops, testing harvests resulting from the use of large, medium and small

seeds, equal numbers of seeds being sown in each case on equal areas. Averaging the results per acre for the whole period he found a marked superiority in favour of the large grains, amounting to as much as 19·1 per cent. for grain crops, 40·3 per cent. for rape, and 60·1 per cent. for root crops. Much variation occurred between the individual crops, as among the cereals oats gave the greatest average increase of 33 per cent., and spring wheat the least increase of 16·5 per cent. over that obtained with small seeds. Less variation appeared among the root crops, all those tested showing marked improvement from the use of large seed. The results are sufficiently striking to merit reproduction.

Table I. *Zavitz's results with Field Crops.*

Equal numbers sown with all sizes of seed on equal areas.

	Crop	No. of years' tests averaged	Yield per acre		
			Large seed	Medium seed	Small seed
			Bush.	Bush.	Bush.
Grain	Oats	7	62·0	54·1	46·6
	Barley	6	53·8	—	50·4
	Spring wheat	8	21·7	—	18·0
	Winter wheat	6	46·9	—	40·4
	Field peas	6	28·1	—	23·0
			Tons	Tons	Tons
Roots	Mangels	5	33·2	29·6	21·5
	Sugar beets	5	22·9	21·9	14·3
	Swede turnips	5	17·1	15·2	8·7
	Fall turnips	4	25·4	21·7	16·2
	Field carrots	5	24·5	22·2	16·2
Rape	Rape	5	17·4	15·0	12·4

In this connection wheat has been repeatedly tested, especially in the United States. Thirty years ago Georgeson and his collaborators (8, 9) arrived at the conclusion that there was no doubt of the advantage of sowing seed of heavy weight per bushel, their average results for three years' trials with wheat showing an increase of two bushels per acre of grain, with a possible slight increase in straw production. The light grade seeds are light because they contain a percentage of small and shrivelled grains of less vitality than plump grains, and these may fail to germinate or may produce weak plants. Under very favourable conditions, however, a thicker strand may be obtained from light seed owing to the larger number of seeds sown per bushel, and the difference from the crop obtained from heavy seed may thereby be diminished. For instance, in 1896, Zimmerman wheat of rather poor quality was sown on rich soil (10), and

a wet season caused a heavy growth and large yield, which was practically the same from both light and heavy seeds. Hays (11) and his fellow workers emphasised the distinction to be drawn between size and weight of seed in estimating crop results. They practised a system of continuous selection of seed, not merely a yearly selection from the bin as other workers had often done. The actual size of seed seems to be of less importance, as the largest kernels gave poorer yielding varieties than the smallest kernels, but heavy hard kernels produced varieties yielding nearly two bushels per acre more than the largest kernels, the experiment therefore emphasising the importance of selecting seed wheat of high quality and heavy weight. More recently Wheeler and Balz (18) have again obtained an increase of over two bushels per acre in favour of heavy seed in wheat, equal volumes of differing weights per bushel being sown. The heavier seed came up the earlier with a stronger and more vigorous plant, and it stooled out the quicker. Later on the conditions were very favourable for a good grain crop and the plants from the lighter seeds made up some of the early differences, but even so in seven trials the heavier seed gave the bigger crop in every case but one. Occasionally the light seed gave heavier straw but lighter grain, due to the crop from the lighter seed being later and not filling out so well as that from better seed. Other things being equal, the earlier wheat is, the better it fills in an average season. Desprez (5) also advocates sowing large heavy seed wheat, at the same rate by weight per unit area as would be necessary with small light seed. He quotes experiments made at the Station agricole de Cappelle with five varieties of wheat, in which equal areas were seeded with similar weights of large and small grains. In every case a heavier yield of grain was obtained from the larger seed, ranging from 4 to 23 per cent. increase according to the variety, and with three varieties the weight per hectolitre was also heavier, in one instance that from heavy seed being 81·6 kilos. and from light seed 78·4; the other two varieties showed no difference. The yield of straw was sometimes to the advantage of the heavy seed and sometimes of the light, again according to variety.

Bolley (3) conducted controlled tests with a definite number of seeds of known weight. Plump seeds of Scotch Fife were used, 800 weighing $23\frac{1}{2}$ gms. and 800 weighing $12\frac{1}{2}$ gms. being sown one and a half inches apart in rows. The total weight of straw and grain was over 10 per cent. in favour of the heavy seed, and the weight of the heads alone over 12 per cent. in the same direction.

With wheat, therefore, it seems evident that under normal or unfavourable conditions of growth the use of well filled, heavy seed results

in a definite increase of crop, frequently about two bushels per acre, but that in seasons very suitable to the growth of cereals this benefit is not always obtained.

Voelcker(16), on the other hand, carried out pot cultures at Woburn to compare the crops obtained from sowing "head" and "tail" corn of wheat and barley, and found no advantage from the use of the heavier seed. This may have been due to the fact that the plants were grown under very advantageous conditions, thus bearing out the general conclusion previously quoted, or possibly a certain shortage of plant food existed in the pots, acting as a limiting factor to growth and tending to level up results in some cases, as no record is given of the application of fertilisers to the soil used.

Zavitz's results with oats, already quoted, confirm the earlier work of Boss(4), who found a decided advantage on the side of heavy seed, far more marked than with wheat. He further stated that his increase of nine and a half bushels per acre, though heavy, was not so great as usual, as under good growing conditions light seeds do better than when conditions are poor, exactly as in the case of wheat.

With barley again, Soule and Vanatter(15) found a constant advantage on the side of heavy seeds. In this case a definite number of seeds were sown per plot, and the large and small seeds were selected for each plot from heads of various sizes, three varieties being tested. Field experiments on the light sandy land at Woburn(17), however, showed no benefit from the use of heavy seeds, rather better crops being obtained from the "tail" than the "head" corn. With maize Georgeson and his fellow workers(7) failed to obtain any advantage from the use of large seed.

Among the leguminous crops, Lehmann(13) with peas, and Fruwirth(6) with lucerne and sainfoin have obtained better crops from the use of heavier seed, though with perennial plants the initial advantage does not persist, but decreases with increasing age of the plant. In experiments on the growth and variability of Helianthus, Reed(14) concluded that plants which were small at maturity were generally small from the beginning, and those which were large at maturity had a well marked superiority from the start.

Illustrations similar to the above might be multiplied[1], but sufficient evidence has been put forward to indicate that in field practice a decided advantage is usually to be obtained by the use of heavy well-filled seed, though under particularly favourable growing conditions the advantage may be decreased, or even, as in the case of wheat, nullified.

[1] Many further references are given by Kidd and West (12).

Two or three years before the publication of Kidd and West's papers on Physiological Predetermination(12) some hitherto unpublished experiments were made at Rothamsted approaching the question of the effect of seed weight from a rather different standpoint. In field tests like those hitherto described, all the plants, whether from light or heavy seeds, are in competition with one another for food, water and light, and also they are subject to bird and insect attacks, which have much effect upon the resulting crop. Furthermore, earlier experiments had been carried out with numbers of selected heavy and light seeds which were bulked together before being weighed, so that little or no information is available as to the progress of events with individual plants grown from seeds of various weights. Experiments were therefore carried out in water cultures with peas and barley, in which the competitive factors were eliminated as far as possible in order that the results could be more closely correlated with the initial weights of the seeds.

A. SEEDS GROUPED BETWEEN KNOWN LIMITS OF WEIGHT.

The seeds were weighed into groups, the difference between the heaviest and lightest in each group being ·01 gm. for barley, and 0·5 gm. for peas. Pedigree strains of seed were used for barley (Pedigree Plumage) and Sutton's Harbinger[1] for peas, and all were selected from one sample in each case. The seeds were germinated in clean sawdust, and as soon as the seedlings were large enough to handle they were transferred singly to 600 c.c. bottles of nutrient solution[2]. Ten plants of each size of seed were reckoned as a unit.

PEAS.

Seeds sown Oct. 16th and put in solutions Oct. 25th, 1915.

Harvested Jan. 25th and Feb. 22nd, 1916.

Two parallel sets were grown and were harvested after 92 and 120 days respectively. The food solution was not changed throughout the experiment, so that all the growth was made at the expense of the initial supply in the nutrient solution, the stock stored up in the seed, and the carbon assimilated from the air. The peas grew from Oct. 25th through

[1] I am indebted to Mr Martin Sutton for the gift of the pea seeds used throughout these experiments.

[2]

KNO_3	1·0	gm.
$MgSO_4$	·5	,,
NaCl	·5	,,
KH_2PO_4	·5	,,
$CaSO_4$	·5	,,
$FeCl_3$	·04	,,

Distilled water to make up one litre.

the winter, and when put into the solutions very little difference was observable between the roots or shoots from large and small seeds. Eighteen days later a regular gradation in size was evident, this being obvious in the roots as well as in the shoots. At this early date there could be no question of a deficiency of food and the greater growth from the heavier seeds would seem to indicate a capacity for making better use of the available food supply at an early stage, as the extra food stored up in the seed is probably more readily available than that resulting from photosynthesis in the young plants. A slight initial advantage obtained in this way would have a considerable effect upon the after growth by the working of the compound interest law, as has been pointed out by V. H. Blackman(1). After 13 weeks' growth the dry weights of the plants showed the same steady gradation as was observed by inspection of the growing plants, the proportionate rise being similar in the shoots and the roots (Table II and Fig. 1). It is often stated that this increase in weight is due to the extra food supplied in the seed. Although this statement is true in effect it is rather misleading, because the extra dry matter formed by the plants from the heavier seeds is in excess of the extra supply in the seeds, the difference in weight of the plants from the lightest and heaviest seeds being in this case twice the difference in the weight of the seeds. A truer view of the matter is that the larger store in the seed enables the plant to make a more rapid initial growth which gives it an advantage that persists in after life.

A month later, after 17 weeks' growth in the original nutrient solution, the difference between the plants from the light and heavy seeds was still very marked, though the discrepancy was not quite so large as at the earlier date (Table II and Fig. 1). Reckoning the weight of the plants from the lightest seeds as 100, after 13 weeks the ratio of the weight of the heaviest to the lightest was 225 : 100, and after 17 weeks only 167 : 100. This probably indicates that the larger plants had almost exhausted their food supply and were growing less rapidly, while the smaller plants had more available food left and were utilising it, thus decreasing the comparative advantage gained by the larger plants. The actual difference between the weights of the large and small plants increased with age, becoming constantly greater than the difference between the original weights of the seeds, thus affording additional proof of the hypothesis that the heavier seeds give the plants a certain initial advantage which makes itself felt throughout life. Growth during the first 92 days was very slow, being in the depth of winter when the light intensity was much depressed, and when harvested the plants from even

Table II.

Peas. Sown Oct. 16th, 1915. Averages of ten plants.

Weight of seed gm.	Grown for 92 days: Total dry weight gm.	Shoot/root	Effic. index	Grown for 120 days: Total dry weight gm.	Shoot/root	Effic. index
Under ·1 gm. (1 plant only)	[·174	4·26	·9148]	[·398	6·22	1·391]
·1 –·15	·344±·005	4·23±·129	1·10	·955±·032	4·85±·15	1·695
·15–·2	·387±·010	4·49±·166	·8628	·990±·029	5·01±·13	1·444
·2 –·25	·470±·012	3·85±·106	·8008	1·144±·027	4·90±·08	1·355
·25–·3	·543±·015	4·21±·111	·7395	1·254±·038	4·82±·12	1·273
·3 –·35	·693±·014	4·28±·174	·823	1·464±·022	4·03±·14	1·254
·35–·4	·776±·012	4·05±·084	·7904	1·596±·030	3·87±·06	1·207

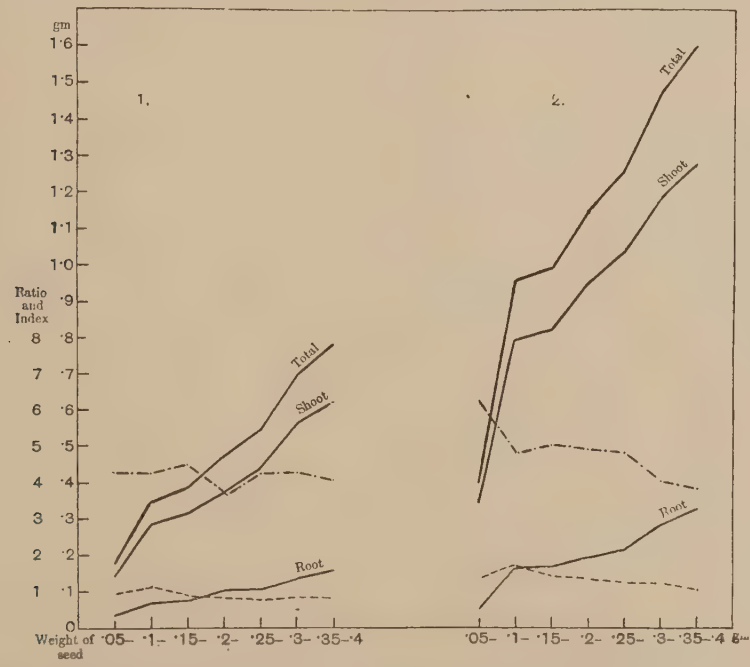


Fig. 1. Harbinger Peas grown from groups of seeds increasing in weight by ·05 gm. in each group. Averages of 10 plants. 1. Oct. 16th, 1915–Jan. 25th, 1916 (92 days). 2. Oct. 16th, 1915–Feb. 22nd, 1916 (120 days). Solutions not changed.
———— Dry weight. —•—•—•— Shoot/root ratio. – – – – – Efficiency index.

the heaviest seeds could not have made very considerable inroads on their supply of food. Nevertheless, the dry weight of the largest plants differed from that of the smallest by four times the difference in seed

weight. Under these circumstances there was no significant difference in the rate of growth of the shoot and root, demonstrated by the shoot/root ratio. The efficiency index, too, was very similar in all cases, though it was possibly a trifle higher with the very light seeds. After longer growth, under improving conditions of illumination, the plants from the very heavy seeds grew so much more than the others that they depleted the nutrient solutions to a greater extent and in this case the roots from the heaviest seeds continued to grow more rapidly than the shoots, thus depressing the shoot/root ratio. The efficiency index (excluding that of the single plant from a seed below ·1 gm.) gradually fell as the weight of the seed decreased. The possible correlation of this fall in the shoot/root ratio and the efficiency index with limited food supply could not be worked out at this stage, but will be discussed later in connection with further data that have since become available (see pp. 236–8).

Barley.

In the first experiments with peas a definite limiting factor was at work in that a restricted amount of food was supplied for the whole period of growth. The action of this limiting factor was investigated in barley by the observation of plants which received a restricted and an abundant supply of food, grown for 67 and 162 days respectively. In the former case the results resembled those with peas under similar conditions, *i.e.* the heavier the seed, the greater the ultimate growth. With barley the difference was more strongly marked, as after only nine weeks' growth the difference in weight of the largest and smallest plants was nine times the difference in the initial weight of the seeds (Table III and Fig. 2). With an abundant food supply (*i.e.* when the food solution was constantly renewed) and a longer period of growth the barley reached maturity, forming ears and ripening seed before harvesting. In the earlier stages of growth steady gradation in size was apparent according to the weight of the seed, but gradually this became less visible, though the difference was still marked if the plants from the lightest seeds were placed alongside those from the heaviest. By harvest time little difference could be observed, as the plants were so large that minor variations were obscured. The dry weights, however, showed that some difference still existed, as the heaviest plants were still obtained from the heaviest seeds. The roots had ceased to show any difference, and the increased weight was manifest only in the shoots, but the mean difference between the lightest and heaviest plants was over 60 times the difference between the initial weights of the seed. The relative growth of shoots and roots, as

Table III.

Barley. Sown Feb. 22nd, 1916. Average of ten plants.

Weight of seed gm.	Solutions never changed. Grown for 67 days: Total dry weight gm.	Shoot/root	Effic. index	Solutions frequently changed. Grown for 162 days: Total dry weight gm.	Shoot/root	Effic. index
·01–·02 (1 plant only)	—	—	—	[27·50	6·65	4·637]
·02–·03	·821±·056	1·949±·084	5·213	29·26±·71	4·895±·043	4·327
·03–·04	·848±·038	1·790±·046	4·758	28·00±·31	5·451±·192	4·127
·04–·05	1·029±·045	2·036±·088	4·671	28·45±·24	5·107±·210	3·981
·05–·06	1·190±·055	1·997±·072	4·588	29·94±·60	5·397±·116	3·889
·06–·07	1·270±·037	2·597±·104	4·436	30·75±·60	6·151±·263	3·803

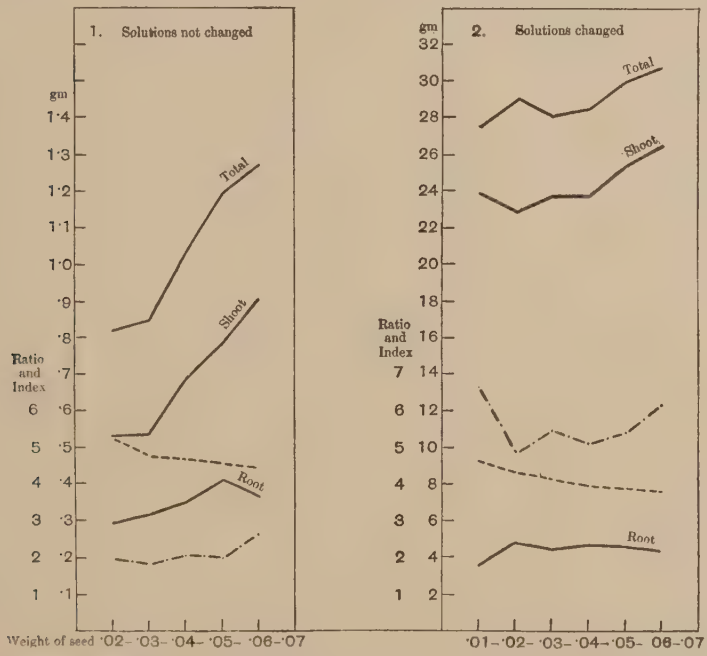


Fig. 2. Barley grown from groups of seeds increasing in weight by ·01 gm. in each group. Averages of 10 plants. 1. Feb. 22nd–April 29th, 1916 (67 days). 2. Feb. 22nd–Aug. 2nd, 1916 (162 days).

———— Dry weight. —·—·—·— Shoot/root ratio. – – – – – Efficiency index.

shown by the shoot/root ratio, was little affected except in plants from the heaviest seeds, in which it tended to rise, the actual value being influenced by the scarcity or abundance of food supplied. The efficiency index under both sets of conditions fell slowly with increasing weight of

seed (see p. 238). The change in the apparent gradation in the size of plants with age was also illustrated by the quantity of nitrate used up by the plants at different periods.

Table IV.

Potassium nitrate utilised by barley plants.

	Weight of seed gm. →		·01–·02	·02–·03	·03–·04	·04–·05	·05–·06	·06–·07
	Nitrate used in — days	After-days previous growth	Solutions frequently renewed					
			gm.	gm.	gm.	gm.	gm.	gm.
Ap. 18	7	35	·335	·425	·51	·58	·629	·717
May 15	6	63	·885	·96	·945	·977	·963	·965
May 21	5	69	·87	·905	·852	·915	·96	·963
May 26	6	74	·73	·848	·865	·945	·882	·907
			Solutions never renewed					
Ap. 19	43	—	—	·545	·595	·695	·75	·75
May 1	55	—	—	·712	·725	·783	·83	·847

The solutions were changed weekly or oftener, and analyses were frequently made of the nitrate remaining in them at the time of renewal, 1 gm. per litre being supplied on each occasion. For the first few weeks the nitrate used up by the smaller plants was considerably less than that by the larger, a gradation occurring according to the size, twice as much nitrate being absorbed by the largest plants as by the smallest. As time went on the difference became very much less, all plants utilising the nitrate freely, though those from the lightest seeds absorbed appreciably less to the end of the experiment. When the nutrient solutions were not renewed, thus limiting the available food supply, the nitrate was never so thoroughly exhausted from the solution even by the plants from the heaviest seeds, but these still utilised considerably more than those from the lighter ones (Table IV).

In the experiments considered, where the food solution was not changed, the length of the period of growth was determined by the food supply, as it was necessary to harvest the crops before starvation deteriorated or killed the plants. Consequently, the peas formed but few pods, if any, and the barley was cut before the formation of ears, and under these circumstances the effect of the size of the seed upon growth was strongly marked, especially in peas. The weight of seed may therefore be of special importance in the case of crops that are normally cut before reaching maturity, being grown for their leaves rather than for fruit and seed.

B. SEEDS OF KNOWN INDIVIDUAL WEIGHT.

PEAS.

The results obtained with the grouped seeds raised the question of how the plants from individual seeds of known weight would behave in respect to total growth and rate of increase. A number of Sutton's Harbinger peas were therefore accurately weighed and experiments were carried through in the early autumn and in the spring, at times when good normal growth was to be expected. The seeds were germinated in damp sawdust, being kept separate by pieces of cardboard recording the initial weights of each seed. As soon as large enough the seedlings were transferred to water cultures and divided into three sets, each set running up the scale from the lowest to the highest weights. In two sets the solutions were constantly renewed, but in the third no fresh nutrients were supplied, distilled water only being added as necessary. When the unchanged set showed signs of suffering from lack of food the plants were harvested, together with one set growing in the changed solutions, the others being allowed to grow on for some time longer before cutting. Two experiments were carried out, but the results were generally so similar that they may be considered together, the figures given being those of the autumn test:

Seeds sown Aug. 31st, ranging in weight from ·095–·421 gm.
Put in solutions Sept. 8th.
Harvested Nov. 16th (solutions changed and unchanged).
„ Dec. 8th (solutions changed).

When put into solutions the plants from the heavier seeds were distinctly ahead of those from the lighter both in shoots and roots. At an early stage the roots of the larger plants developed strong sturdy rootlets, which were thickly placed down the main roots and were closely studded with laterals in their turn. Those from the lighter seeds developed much thinner rootlets which were spaced further apart on the main root and were not so well supplied with laterals themselves. These phenomena occurred as a *general tendency* and could not, at this stage at least, be interpreted as representing a fixed rule in the gradation of plants from light or heavy seeds. In the earlier stages of growth, the leaves from heavy seeds were bigger than those from light ones. At all the harvests the appearance resembled that obtained in the earlier experiments, the general gradation of large to small plants from heavy to light seeds being clearly evident. Each plant was harvested individually, and the total dry weight and the efficiency index of each obtained. The results are graphed in

Figs. 3 and 4, but for considerations of space the actual figures are not listed. In this connection attention should be drawn to the fact that in all these experiments with weighed seeds the efficiency index is perforce rather too low. It was naturally impossible to find out the initial dry weight of each seedling when put into the solution, as that would have entailed its destruction, so it was necessary to use the original weight of the seed for W_0 in calculating the efficiency index. Other experi-

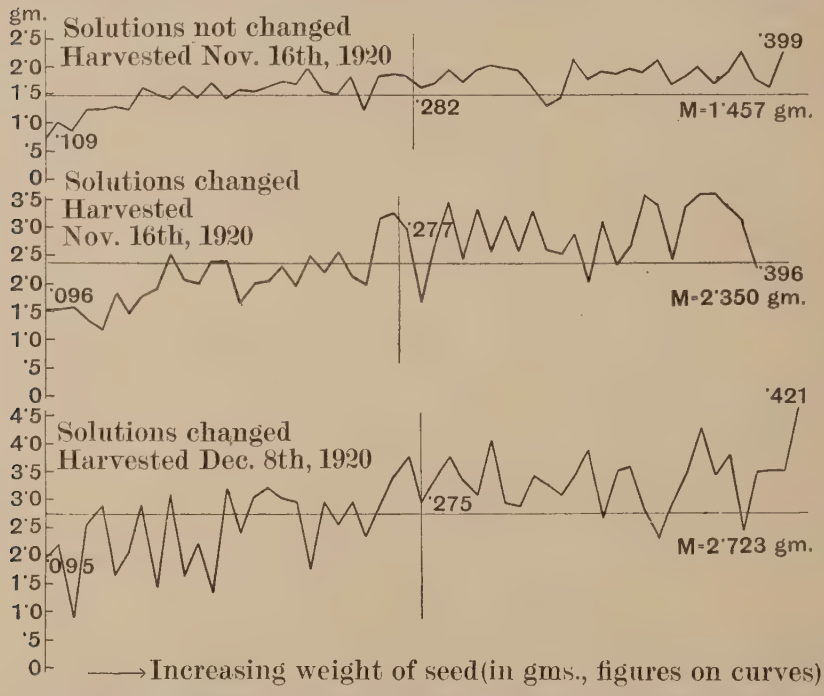


Fig. 3. Dry weights of three series of Harbinger Peas grown from seeds of known individual weight, sown on Aug. 31st, 1920. The line *M* indicates the mean of the highest and lowest dry weights in each series. The vertical lines divide the series into two equal parts.

ments(2) have shown that at this stage the seed has lost a good deal of its original weight by respiration and that this has not yet been made up by photosynthesis, so the initial weight (W_0) used is rather too high and tends to reduce the value finally obtained for the index.

In the presence of a limited supply of nutrients flowering was retarded with all the plants and few pods were produced, those that did form being small and undeveloped. With plenty of food all flowered freely, but on the smaller plants the pods remained small and poorly developed, whereas

on the larger plants they filled out well. The gradation between the plants from light to heavy seeds was very marked both in shoots and roots, especially where unlimited food was available, and this was borne out by the dry weights at harvest. These showed considerable variation in plants from seeds of much the same weight, but the trend of the curve was always upwards. This is well shown if the mean dry weight of the lightest and heaviest plant in any set be taken as a datum line and the

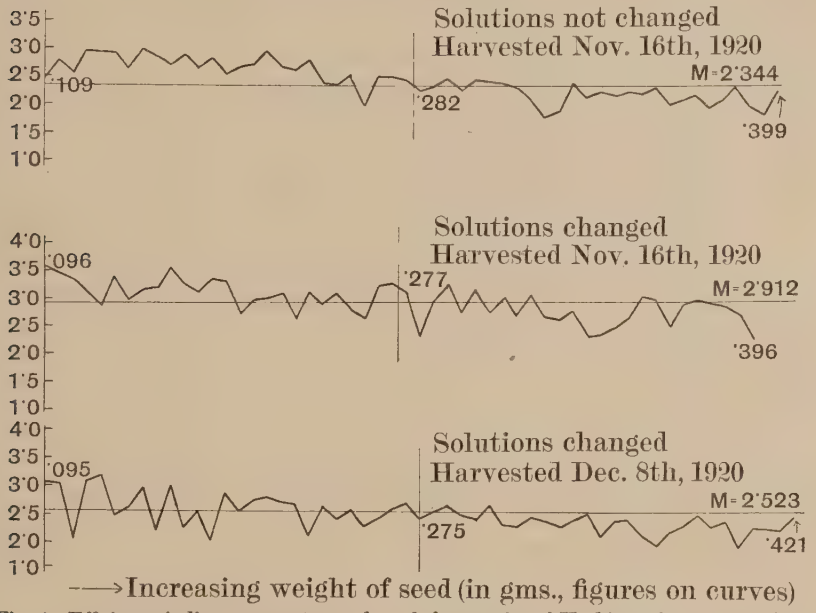


Fig. 4. Efficiency indices per cent. per day of three series of Harbinger Peas grown from seeds of known individual weight sown on Aug. 31st, 1920. The line *M* is drawn for purposes of comparison at the mean of the highest and lowest efficiency indices in each case. The vertical lines divide the series into two equal parts.

plants from the lighter and heavier seeds compared with it. For instance, in one case (see Fig. 3, 2nd curve), where mean dry weight was 2·350 gms.,

Among plants from 26 lightest seeds (·096–·268 gm.)

7 dry weights were above mean, and 19 below.

Among plants from 26 heaviest seeds (·277–·396 gm.)

22 dry weights were above mean, and 4 below.

Similar figures could be shown for every set.

If, however, the peas were grown on for some time and allowed to approach maturity, it was found that above certain weights the advantage of the heavier seed became less, though it was still very marked among

the plants from the lighter seeds. This is best seen by grouping the plants into quartiles and striking the mean dry weight in each; this rises steadily and rapidly with the weight of seed except in the last quartile, in which comparatively little advantage appears on the side of the heaviest seeds (Fig. 5 and Table V). This indicates that after a certain stage the initial dry weight of the seed ceases to have any influence upon the resulting crop, at least so far as plants derived from reasonably heavy seeds are concerned. With an annual crop such as peas, natural death occurs soon after this is manifest, while the advantage of heavy seeds over light is still very marked, but it is quite conceivable that with perennial plants the process might continue down the scale until ultimately, after various periods of time, the plants from light seeds were as good as those from heavy ones. In any case with short lived annual plants like peas and barley, there is an obvious advantage to be gained from using heavy well-developed seeds. An explanation of the change in the relative advantage of the seed with age is evident when the efficiency indices are examined (see Figs. 1, 2, 5). In every case, whether with limited or abundant food supply, or whether with a short or long period of growth, the rate of increase of dry weight per cent. per day with peas and barley slowly decreases as the initial weight of the seed increases. The initial capital, represented by the seed, is so much greater in the case of the heavy seeds that even with this lower rate of increase the total weight reached at compound interest for a long period of time rises much above that attained by lighter seeds with a rather higher efficiency index. Eventually, however, the difference in the rate of increase gradually begins to tell, and plants from seeds that were not much below the heaviest in weight pull up into line and become as heavy and well-developed as those that hitherto were their superiors. If healthy growth continues without death supervening, the process goes on, and still lighter seeds produce plants equal to the best. It is, of course, only hypothesis at this stage to assume that this would go on indefinitely until all initial advantage from weight of seed were wiped out, but if it be true, it affords a reasonable explanation of the results of Fruwirth, supporting his contention that with perennial plants the initial advantage of heavy seeds disappears with age. The lower rate of increase with the heavier seed cannot be attributed to any failure in the food supply, as when plenty of food was given the solutions were renewed so frequently that comparatively little of the nitrogen was removed (usually not more than 20 per cent.) before a full supply was again offered. It would rather seem to be due to a gradually increasing inability of the embryonic shoot and

Table V.

Peas sown Aug. 31st, 1920.

Results from about 56 plants in each case, grouped into quartiles of 13 or 14 plants and averaged up.

Mean weight of seed gm.	Total dry weight gm.	Shoot/root	Efficiency index
	Solutions not changed. Grown for 77 days		
·154	1·295±·051	5·24±·11	2·75
·240	1·622±·035	4·85±·09	2·51
·318	1·747±·041	4·63±·14	2·22
·373	1·853±·034	4·47±·08	2·07
	Solutions changed. Grown for 77 days		
·145	1·766±·073	6·79±·31	3·24
·230	2·298±·086	7·33±·25	2·95
·309	2·763±·070	8·33±·25	2·82
·364	2·949±·104	7·81±·19	2·69
	Solutions changed. Grown for 99 days		
·149	2·123±·13	8·51±·31	2·64
·235	2·843±·09	9·02±·25	2·51
·316	3·310±·07	10·22±·26	2·37
·373	3·358±·12	9·15±·28	2·20

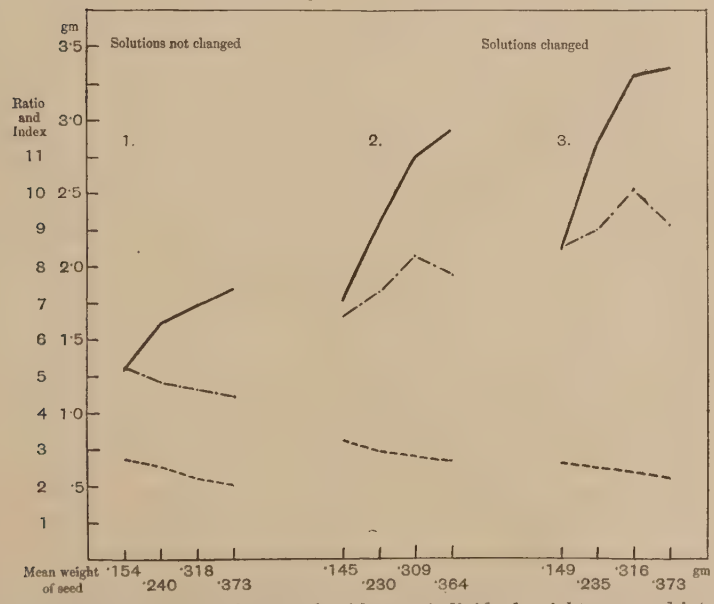


Fig. 5. Harbinger Peas grown from seeds of known individual weights grouped into quartiles. Mean figures for 13 or 14 plants in each case. 1, 2. Aug. 31st–Nov. 16th, 1920. 3. Aug. 31st–Dec. 8th, 1920.

——— Total dry weight. —·—·—·— Shoot/root ratio. – – – – – Efficiency index.

root to make the fullest use of the extra supplies of nutriment presented with the heavier seeds. In other words the improvement in the growth capacity of the young shoot and root with increasing size of seed is not fully commensurate with the additional food supplies laid down in the cotyledons or endosperm, and this is reflected in a slightly lower rate of working as revealed by the decreased efficiency indices.

A comparison of plants from the same sowing, grown under similar conditions with plenty of food, but harvested at different times shows that the efficiency index per cent. per day of plants from the same size of seed decreases as time goes on (see Fig. 5, curves 2 and 3). This is in full accord with the earlier experiments(2) which showed that the efficiency index over weekly periods first rises to a maximum and then falls, so that the average rate of increase over the whole period is less than that attained in the earlier part of the life of the plant. The relative development of shoot and root are to some extent influenced by the initial weight of the seed, the effect partly depending on the abundance or scarcity of food. With barley grown with a limited food supply for 67 days the ratio between shoot and root was about 2, whatever the weight of the seed, both shoot and root developing at equal rates except with specially heavy grains weighing over ·60 gm., when the ratio rose to 2·6 (Fig. 2). With plenty of food for 162 days the ratios for medium sized seeds approximated to 5–5·4 (omitting the single plant from one seed below ·02) rising to over 6 with very heavy seeds. The rise in the ratio with the improved food supply is significant, indicating that when deficiency of food is not a limiting factor the shoot of barley tends to increase more rapidly than the root; this is chiefly evident when the external source of supply is abundant, but is shown to some extent when the reserves stored in the endosperm are large, as occurs with very heavy grain. Peas, on the contrary, gave quite different results. With a limited supply of food the shoot/root ratio steadily decreased as the seeds became heavier, under such conditions the roots being able to make better progress than the shoots. When, however, plenty of food was available the ratios showed a corresponding rise, greater aerial growth being made with a more limited root system, probably indicating a greater assimilatory activity in the plants from the heavy seeds. This again was followed by a decrease with the heaviest seeds, no corresponding decrease being shown with barley (Fig. 5). The pea plants in the latter case were not very much heavier than those from the next grade of seed, especially after prolonged growth, and the fall in the shoot/root ratio suggests that the advantageous effect of the heavier seed persists in the root after it has ceased to tell

with such effect on the shoot. This is borne out by the fact that the fall is more strongly marked after a longer period of growth (cf. plants harvested on Nov. 16th and Dec. 8th, Fig. 5). As time goes on the shoot/root ratio increases, but the rise in the plants from the heaviest seeds is considerably less than in those from rather lighter seeds.

CONCLUSIONS.

The results thus obtained with peas and barley grown under controlled conditions from seeds of different weights, go to support the view of the advantage generally to be gained with crops that are short lived, as spring cereals, roots, peas and beans, by sowing heavy well filled seeds instead of corresponding numbers of light seeds even though equally well filled. The data obtained from these experiments do not show the comparative results of sowing equal volumes of the two classes of seed, *i.e.* equal number of bushels per acre.

The results further indicate that with a longer growing period the advantage tends to diminish owing to the less rapid rate at which the percentage increase of dry weight takes place with the heavier seeds, thus providing an explanation of the field results with perennial crops such as lucerne, in which the weight of the individual seeds sown is apparently immaterial. There is every justification, therefore, for laying emphasis on the importance of the quality and weight of seed, as larger crops may be anticipated from the use of larger and heavier seeds, more especially if the season is unfavourable, though even initially poor plants may make very satisfactory progress if the season is exceptionally favourable to the growth of any crop.

SUMMARY.

(1) Field experiments, especially in America, have indicated that, with annual plants at least, better crops are usually obtained by the use of heavy seed in preference to light, whether equal numbers of bushels or equal numbers of seeds are sown per acre.

(2) Water culture experiments with peas and barley have shown that there is a steady and considerable rise in the dry weight of the plants as the initial weight of the seed increases. This occurs both with a limited and abundant food supply.

(3) The efficiency index (rate per cent. increase per day) falls gradually as the weight of the seed rises. With prolonged periods of growth this tends ultimately to counterbalance the initial advantage gained by plants from the heavier seeds, but with annual crops as cereals, peas, etc.

harvesting occurs before this equilibrium is reached, leaving the advantage with the heavier seeds.

(4) The relative development of shoot and root is to some extent influenced by the initial weight of the seed, but may vary with the species and with the amount of available food.

(5) The results lend support to the growing agricultural practice of advocating the use of large heavy seed, especially with annual crops. The advantage in the case of perennials would appear to be less, if any, but this has not been determined by laboratory experiments.

REFERENCES.

(1) Blackman, V. H. (1919). *Ann. Bot.* xxxiii, 353–360.
(2) Brenchley, W. E. (1920). *Ann. App. Biol.* vi, No. 4, 211–244.
(3) Bolley, H. L. (1901). *Eleventh Annual Report*, North Dakota Agric. Exp. Stat.
(4) Boss, A. (1893). Minnesota Agric. Exp. Stat. *Bull.* 31, p. 213.
(5) Desprez, F. (1922). *Journ. Agric. Pratique*, T. 1, No. 7, pp. 141–143.
(6) Fruwirth, C. (1917). *Fehling's Landw. Ztg.* lxvi.
(7) Georgeson, C. C., Burtis, F. C. and Shelton, W. (1891). Kansas Exp. Stat. *Bull.* 30, p. 187.
(8) Georgeson, C. C., Cottrell, N. M. and Shelton, W. (1891). *Ibid. Bull.* 20, pp. 7–9.
(9) Georgeson, C. C., Burtis, F. C. and Otis, D. H. (1893). *Ibid. Bull.* 40, pp. 55–57.
(10) —— (1896). *Ibid. Bull.* 59, pp. 98–99.
(11) Hays, W., Hoverstad, T. A., Pendergast, W. W. and Boss, A. (1896). Minnesota Agric. Stat. *Bull.* 50, pp. 322–325.
(12) Kidd, F. and West, C. (1918–19). *Ann. App. Biol.* v, pp. 1–10, 112–142, 157–170, 220–251; vi, 1–26.
(13) Lehmann, J. (1869). *Zeitschr. d. landw. Vereins in Bayern.*
(14) Reed, H. S. (1919). *Amer. Journ. Bot.* vi, 252–271.
(15) Soule, Q. M. and Vanatter, P. O. (1901). Tennessee Agric. Exp. Stat. xiv, *Bull.* 3, pp. 3–6.
(16) Voelcker, J. A. (1901–2). *Reports on Field and Pot Culture Experiments*, Woburn Experimental Station, pp. 48–51; 1902, pp. 34–35; 1903, pp. 29–30.
(17) —— (1905–6). *Ibid.* pp. 22–23.
(18) Wheeler, W. A. and Balz, S. (1906). South Dakota Agric. Coll. *Bull.* 96, pp. 59–60.
(19) Zavitz, C. A. (1905). *Ann. Rep.* Ontario Agric. Coll. and Exp. Farm, pp. 170–172.

(*Received December 6th*, 1922.)

NOTES ON THE CONTROL OF "CUTWORMS" BY POISONED BAIT

By J. C. F. FRYER, M.A. and R. STENTON.

(*Ministry of Agriculture.*)

(With 3 Text-figures.)

Abroad, and notably in the U.S.A., the destruction of Agrotid caterpillars (Cutworms) by means of a poisoned bait is generally recognised as the standard treatment, but in Britain, although entomologists appear to have used the method from time to time, the published records of their results are too scanty to justify any final conclusions. The abnormal abundance of Agrotid larvae in August and September of 1921 gave an opportunity for carrying out a further series of experiments which are here described, chiefly for the information of entomologists, since it is recognised that there are still difficulties to surmount before the method can be advised to the farming world in general.

The use abroad of poisoned bait against Cutworms was the direct outcome of its success in dealing with plagues of locusts or grasshoppers and it would seem that in the majority of cases the same mixture has been used. Little information is, however, available as to the precise part played by some of the constituents and it seemed wise, therefore, before proceeding to field trials to discover in the laboratory whether the Cutworms (*Euxoa* (*Agrotis*) *segetum*) had any preference for any particular chemicals or flavours. The American bait consists of moist bran as a base, flavoured with cane molasses and lemon, and rendered poisonous with Paris Green. Since moist bran in practice is a very convenient base it was retained in these experiments and when flavoured with one of the substances under trial, was tested according to one or other of the following methods.

Series I.

The floor of a cage 2 ft. square was divided by wooden partitions 1 in. high into four shallow compartments of equal size. The compartments were filled with peat moss litter so as just to bury the partitions and three different substances under test were placed one in each com-

partment in the corner furthest from the centre (Fig. 1). In the corresponding corner in the fourth compartment was placed a potato or mangold in accordance as to whether the larvae under test had been obtained from a potato or a mangold field. From 50 to 100 larvae were then allowed to bury themselves in a shallow dish completely filled with peat litter, which was placed exactly in the centre of the cage floor resting on the intersection of the four partitions and a little further peat was sprinkled round the outside of the dish so as to make it possible for the larvae to crawl out easily. As Agrotids are chiefly nocturnal feeders the cage was left for the night, care being taken to prevent external influences such as light or wind from affecting any one side more than the other. The following morning the number of larvae in each compartment was counted, the assumption being that after feeding the larvae would burrow into the peat upon which the food rested and would be prevented by the partitions from straying into one of the other compartments in which they had not fed. It was also assumed that, as the natural food was always present, the larvae would not feed on the baits unless they were at least as attractive.

The results from three of these experiments were as follows:

(The figures represent the numbers of larvae, reduced to percentages, found in the compartment in which the particular substance named had been placed.)

Exp. 1.	Natural food (mangold)	43 per cent.
	Citric acid and moist bran	22 ,,
	Amyl acetate and moist bran	22 ,,
	Molasses and moist bran	13 ,,
2.	Natural food (mangold)	18 ,,
	Molasses and moist bran	29 ,,
	Chopped turnip leaves	31 ,,
	Amyl acetate and moist bran	22 ,,
4.	Natural food (mangold)	19 ,,
	Molasses and moist bran	26 ,,
	Chopped turnip leaves	30 ,,
	Amyl acetate and moist bran	25 ,,

A number of similar experiments were made, but they need not be reported as no deductions could be made from them except that the larvae appeared to show no very marked preference. It seemed to be more or less a matter of chance whether they fed upon the bran bait or upon their natural food plant.

A fresh type of cage was then evolved to deter still further the larvae from wandering, since it was considered that the results obtained in the first series of experiments might have been vitiated by the movement of larvae after they had fed.

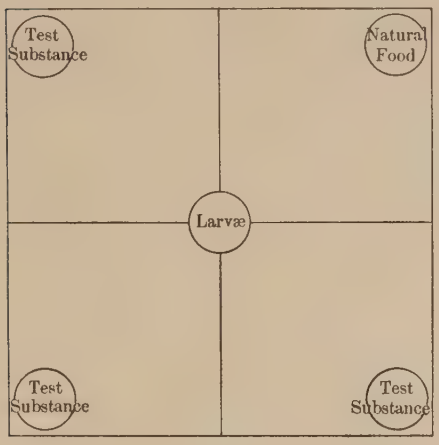


Fig. 1.

Series II.

A cheese tub was fitted with a central partition dividing the tub into two compartments with the exception of a space of about 2 ins. at each end of the partition (Fig. 2). The whole floor was covered with peat and

Fig. 2.

the natural food placed on one side of the partition near the centre, the moist flavoured bran in a similar position on the other. Equal numbers of larvae were placed in each of the two compartments and the experiment was left until the following morning, when the numbers of larvae

in the compartments were counted. In this instance it was assumed that if a larva was content with the food in its own compartment it would make a meal and when satisfied remain near by; that otherwise it would wander to the opposite compartment in search of food more palatable. It was believed, for instance, that unless a larva was satisfied with "flavoured" bran, it would go in search of mangold or turnip before making its nightly meal.

The following results are typical of those obtained by this series:

Exp. 6.	Turnip	73 per cent.
	Clove oil and moist bran	27 ,,
9.	Turnip	50 ,,
	Beet molasses and moist bran	50 ,,
12.	Mangold	52 ,,
	Molasses, colza and moist bran	48 ,,
14.	Mangold	60 ,,
	Colza and moist bran	40 ,,

In the case of clove oil a definite aversion could be deduced but otherwise the larvae again appeared to exhibit no strong preference.

At this point it may be well to state that some of the larvae which had been discovered on the "bran" side in the various experiments were dissected and all showed evidence of having actually fed upon the bran.

Series III.

In view of the ambiguous results of these two series of experiments a third form of cage was designed in order to impose on the larvae the necessity of choosing definitely between their own food plant and the bran bait. The apparatus in this case again consisted of a cheese tub but with a high partition passing from the circumference of the tub diametrically to within 6 ins. of the side (Fig. 3). Opposite the free end of the partition a small compartment about 4 ins. square was constructed with a narrow opening directly facing the partition end. The tub was thus divided into three compartments, two large and one small, intercommunicating by a narrow space at the free end of the main partition. A thickness of 3 or 4 ins. of peat litter was then placed on the floor of the tub and on one side of the main partition a mangold, potato, etc. according to the field from which the larvae under test were obtained. On the other side a handful of the flavoured bran mixture was partly buried in the peat. A trail of the same bran mixture was then laid from the main mass to the opening of the small compartment or "box," where

it mingled with a trail of chopped mangold leading to the mangold root itself. From 40 to 50 larvae were then placed in the box in peat, in which they buried themselves until evening. On moving to search for food, they could only leave by the opening where they were at once presented with the bait and their own natural food, in such a manner that they could not fail to realise the presence of both. It was assumed, as actually occurred, that they would follow one or other trail until they reached the natural root or the bran bait, where they would complete their meal and go to rest in the peat litter near by. Any tendency to wander after feeding would be likely to cause but a small error, since the chances of larvae finding the relatively small opening between the two main compartments would be few. The likelihood was, however, further reduced

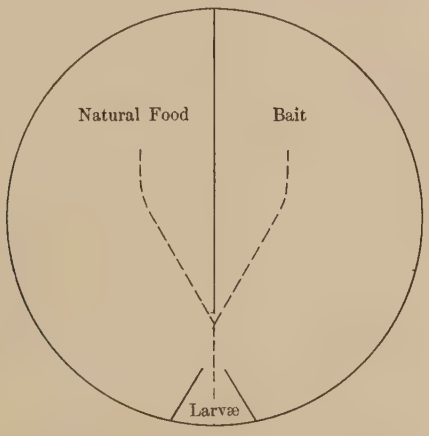


Fig. 3.

by piling peat to a considerable depth at the fixed end of the partition and reducing it so as to be barely sufficient to cover the larvae in the other half of each compartment. Since the tendency of the larvae is to bury themselves, this arrangement of the peat would tend to discourage them from leaving the part of the box in which the food was placed.

The following results were obtained from this form of experiment, the numbers again being reduced to percentages. It may also be mentioned that in most cases a few larvae remained in the box, probably because they had recently fed or because they were changing their skins and therefore unwilling to eat. These larvae are not included in drawing the percentages.

Exp. 7.	Turnip	52 per cent.
	Beet molasses and moist bran	48 „
9.	Turnip	66 „
	Clove oil and moist bran	34 „
11.	Mangold	55 „
	Colza, molasses and moist bran ...	45 „
14.	Mangold	41 „
	Colza and moist bran	59 „
15.	Mangold	53 „
	Moist bran (unflavoured)	47 „
17.	Mangold	47 „
	Moist bran and salt	53 „
19.	Moist bran	82 „
	Peat but no food	18 „
20.	Mangold	85 „
	Peat but no food	15 „
21.	Mangold	61 „
	Pimento and moist bran	39 „
22.	Mangold	53 „
	Mace and moist bran	47 „
23.	Mangold	47 „
	Coconut oil and moist bran	53 „
26.	Mangold	52 „
	U.S. bait: lemon, molasses, bran (moist)	48 „
27.	Mangold	50 „
	U.S. bait: lemon, molasses, bran (moist)	50 „
28.	Mangold	64 „
	Linseed oil and moist bran	36 „
29.	Mangold	57 „
	Dandelion leaves	43 „

In this series of experiments, it will be noted that clove oil (Exp. 9) again showed some signs of being distinctly repugnant and the same was perhaps the case with Pimento (Exp. 21). In the other experiments, except Nos. 19 and 20, preferences are not strongly marked and although in certain cases—Exp. 14—with colza oil, some attractiveness on the part of the oil may be argued, they are either contradicted by other experiments or are not beyond the limits of experimental error. In the case of Exps. 19 and 20 some evidence as to the dimensions of this error are obtained, the figure working out at about 20 per cent. and it is worth noting that this conclusion is supported by the next series of experiments.

Attention may also be drawn to Exps. 26 and 27, where the bait, apart from the absence of poison, was identical with that used in America against Cutworms. It will be noticed that no evidence is given of any special attraction from the use of lemon and molasses, while summarising

the whole series it appears that the addition of flavours does not add to the attractiveness of moist bran, which clearly is not far different from that of the normal food plant.

Series IV.

While the foregoing experiments were in progress, attempts were being made to discover a dye which could be used with the bran, and on being eaten would distinguish the larva in question without causing it material damage and without affecting the attractiveness of the bran bait. It was undesirable that the larvae should be dissected in order to detect the dye, which therefore to be effective had to give a very pronounced coloration to the alimentary canal. Finally, a sample purchased as B.P. liqu. cochineal was found satisfactory and was used in a considerable number of experiments, but it may be noted that after the original sample had been exhausted it proved impossible to obtain a similar dye again. Several dyes made according to the B.P. formula were both purchased and also made up in the laboratory, while innumerable variations were also tested, but none were found to approach the standard set by the first sample, which was indeed almost ideal. A number of aniline dyes were also used with varying results, reds being always the more satisfactory, but none was so generally useful as cochineal, with which this series of experiments was in the main carried out.

In regard to method, a number of experiments with cochineal-dyed bran were conducted with the type of cage described in Series III (Fig. 3) in order to check the error of this apparatus. By counting all the red stained larvae which were found on the side of the partition containing the natural food and by assuming that any unstained larvae found on the bran side had fed upon mangold, etc. (and then crossed over) it was possible to calculate the maximum error, which worked out at approximately 20 per cent. This figure is certainly too high, owing to the inclusion of all unstained larvae found on the side of the cage containing red bran, since a number of such larvae may well have been those which owing to being about to change their skins, were unwilling to eat. Accepting, however, an error of 20 per cent. it will be seen that the conclusions derived from the tests are not substantially affected.

The method was then further developed, so as to approach more nearly the field conditions, by the removal of the main partition in the third type of cage, leaving only the small box-like compartment. Peat to the thickness of 3 or 4 ins. was then laid on the bottom of the cage and potatoes or mangolds buried in it at regular intervals, leaving a

small portion of their surfaces exposed above the peat. Bran dyed red with cochineal, flavoured or not as the case might be, was then sprinkled thinly over the surface of the peat. The larvae, 50 in number, were placed as before in the compartment until the following day, when they were recovered from under the peat and examined to discover which had eaten the bran.

These experiments merely served to confirm the conclusions already formed from previous experiments, to the affect that the larvae were as willing to eat bran as their proper food and that although the addition of various flavouring materials to the former might reduce its attractiveness, in no case did they add appreciably to it. In the experiments with dyed bran about half the larvae showed that they had fed upon bran, while the other half showed no evidence of having done so. It was therefore considered that if a poisoned bran bait could be distributed in a field so that the larvae were as likely to discover it as to find their natural food, then about half the caterpillar population in the field should be killed.

Since at this time the season was advancing, the search for more attractive baits was abandoned, and field trials with moist bran unflavoured but rendered poisonous were arranged. Before giving the results of these trials, a list of the flavouring materials tested may be inserted in order that those who conduct similar experiments may know which were found ineffective. It may also be explained that, apart from the American experiments, there was nothing to suggest what substances were likely to prove effective and the flavouring materials were selected, more or less at random, in the hope of finding something sufficiently marked in attractiveness to justify a detailed investigation and analysis. The time available was, unfortunately, insufficient to enable any organised trials with pure chemicals to be made, and importance was attached to the necessity of a potentially attractive substance being readily available to the cultivator through the ordinary channels of purchase.

Beet molasses	Pimento
Cane molasses*	All spice
Clove oil	Amyl acetate
Colza oil	Allyl isothiocyanate
Linseed oil	Apple chopped
Coconut oil	Lemon juice*
Aniseed oil	Turnip leaves chopped
Cinnamon	Dandelion leaves
Nutmeg	Sodium chloride*
Mace	

Those found effective against Cutworms or Grasshoppers in America are marked with an asterisk.

Series V. Field Trials.

The first trial was carried out on August 22nd at Rothamsted in a mangold field evidently, but not severely, attacked, almost every root showing signs of damage, but comparatively few having been killed. An area of approximately 4 rods was marked out and was dressed in the afternoon with the following mixture:

Bran dyed with cochineal ... ... $\frac{1}{2}$ lb.
Paris Green ... ... $\frac{1}{2}$ oz.

the mixture before use being moistened with water until the particles of bran just failed to adhere to each other. The bait at this stage feels damp to the hand but can still be easily sown broadcast. On a part of the experimental area the bran was sown down the rows and on another it was scattered uniformly, but this difference may be neglected, as subsequently it was found to have no effect on the ultimate result. On the following afternoon a portion of the treated area was examined with great care and every larva found was gathered, in all 68. Of these, four were dead, while 24 showed signs of arsenical poisoning. The four dead larvae proved on dissection to have eaten freely of the poisoned bran. The remaining larvae were kept to discover the numbers which died or recovered. On the day following a similar portion on the plot was searched and produced 60 larvae, of which 15 were dead and 12 evidently poisoned. As before, these were kept until a final result was obtained on August 25th as follows:

1st lot—living, 41; dead, 27.
2nd lot—living, 29; dead, 31.

Summarising, out of a total of 128, 58 were destroyed by the treatment, approximately 45 per cent.

This percentage is probably a little too low, as it is exceedingly easy to overlook dead larvae, which shrivel rapidly on a hot day and are very inconspicuous in the soil or at times even upon the surface, for they were found in either situation.

The measure of success obtained in this small trial and also the fact that the results corresponded so nearly with the probable results as calculated from the laboratory experiment, made it desirable to extend the work to trials under normal farm conditions. This took place at Symonds Hyde, Hertfordshire, first on August 29th and then on September 6th on a field partly mangolds and partly swedes, which was being seriously damaged by Agrotids (practically all *A. segetum*). One

and a half acres of mangolds and one acre swedes were dressed with bran 56 lbs. to Paris Green 3 lbs. moistened with water as in the previous experiment, the amount of water needed being approximately seven gallons. The work was done by farm labourers who sowed the plot broadcast, only covering two and a half acres instead of three as was originally intended. The bait was sown between 4.30 p.m. and 5.30 p.m. summer time, but the day was hot with a drying wind and much of the bait must have become very dry before the night. On August 31st the field was examined, small areas about two square yards in different parts being searched and every larva found removed. No difference in result between the swede and mangold areas was detected and on September 2nd, until which day all the living larvae had been kept, the final result was recorded as follows:

Larvae living	170
Larvae dead	144

The percentage thus again works out at about 45 per cent. killed.

On September 6th a further trial was made, partly to test a mechanical method of distributing the bait and partly to discover if lead arsenate could be substituted for Paris Green. A Paris Green mixture was made up as before, while in the case of lead arsenate 6 lbs. of paste (20 per cent. As_2O_5) were used to each 56 lbs. of bran. Both lead arsenate and Paris Green were applied with a manure distributor of the Disc type (manufacturer, J. R. Wallace) which did the work well and with hardly any clogging. Some four acres in all were dressed as follows: one acre swedes which had previously been dressed on August 29th and one acre swedes not previously treated, both with Paris Green; two acres swedes (not previously treated) with lead arsenate. The field was again visited on the 9th, and as time was limited a collection of larvae was made on two sample areas of four square yards in each plot with the following results:

(*a*) *Swedes twice dressed.*

Larvae living	40
Larvae dead	59

i.e. approximately 59 per cent. of the larvae were found to have been killed.

Much reliance must not, however, be placed upon this figure, for while some larvae had been killed by the second dressing, others had evidently been dead for some days and had succumbed to the first dressing. All of the latter would not, however, be recoverable after so many days, and therefore the figure is too high to represent the results of the single dressing and too low for the two dressings.

(*b*) *One acre swedes once dressed Paris Green.*

Larvae living	75
Larvae dead	40

In this case approximately 35 per cent. of the larvae only were killed, a result which may in part be due to the fact that the collection was made very hurriedly.

(*c*) *Two acres swedes once dressed with lead arsenate.*

Larvae living	91
Larvae dead	8
Larvae poisoned but not killed ...	10

As lead arsenate, at this strength at all events, was obviously ineffective, the ten poisoned larvae were not retained to obtain final figures as to mortality. As a check on the above figures and to show that the larvae were not dying from any natural disease, one of the counts from a control plot, in this case swedes, may be mentioned, the area named being four square yards:

Living, 59. Dead, 0.

At this point the swede and mangold experiments were left, as the damage to the roots had already been sufficiently great to invalidate any results to be obtained by weighing the crop.

On the same farm a field of potatoes had been ruined by the Agrotids and although owing to their great depth in the soil it seemed almost hopeless to deal with them by the bait method, a small preliminary trial was carried out. It proved that a few of the larvae at all events came to the surface and died after eating the bran but circumstances did not permit of the trials being concluded and no figures can be given.

Conclusion.

The foregoing experiments show that the bran bait method of dealing with Cutworms is very fairly effective in the case of mangold and swede fields, one dressing destroying about 45 per cent. (one plot fell to 35 per cent.). The search in the laboratory for substances specially attractive to Agrotid larvae failed to produce anything better than moist bran, which appeared to be taken practically as readily as the natural food, so that on an average 50 per cent. of the larvae tested fed upon bran and 50 per cent. upon their own proper food. The approximation of this figure to the number destroyed in the field experiments is curious and to some extent the two types of experiment support each other. This is explained, not by presupposing a preference for bran by 50 per cent. of the larvae, but by assuming that the larvae have no preference and have an even chance of meeting with bran or their own food.

As regards the future of the poisoned bait method in practical agriculture, the chief difficulty lies in the possibility that the poisoned bran might prove a danger to poultry, game and other birds, or even it has been suggested, to man through eating poultry and game which themselves had been feeding on the bran or poisoned larvae. All those who have experimented in the U.S.A. or Canada have satisfied themselves that this danger is not a real one and the method is now recognised as standard, the only precautions advised being those relating to the mixing tubs or to the labour engaged in distributing the bait. In the experiments previously recorded, the dressing appeared very slight, so that, without a most careful examination, it would be impossible to discover from the bran on the surface which areas had been dressed and which had not. It seemed almost impossible that so small a dressing could prove a danger. On the other hand the amount of Paris Green used per acre was 1 lb. while in America $\frac{1}{3}$ lb. per acre is considered sufficient. There appears to be no evidence to show the quantity of Paris Green which is toxic to vertebrates, but on the assumption that each one grain of arsenious oxide in combination would form a poisonous dose, it would seem that in order to be poisoned a bird would have to eat every particle of bran on $1\frac{1}{2}$ to 2 square yards (or from $4\frac{1}{2}$ to 6 square yards dressed on the American formula). This possibility, though unlikely in the circumstances in which the bait is used, cannot be altogether ruled out and it does not seem wise to recommend the treatment for application in agriculture except in special circumstances. It should also be noted that no opinion is offered as to the conditions under which the use of the bran bait would be an infringement of the Protection of Animals' Act, 1911, Section 8.

On all accounts, therefore, it is evidently desirable that some poison should be discovered which is sufficiently toxic to the larvae but in the quantities used would prove harmless to birds and other vertebrates. The discovery of some such insecticide should not prove impossible.

In conclusion, the authors desire to thank Sir John Russell of the Lawes Agricultural Trust for permission to experiment on a field on the Rothamsted farm, and also to Mr T. G. Walker of Symonds Hyde Farm, for the assistance he rendered both in placing his fields at our disposal and for his generous co-operation throughout the work. Dr Imms of Rothamsted also kindly assisted us in regard to suitable substances for use with the bran.

(*Received September 4th*, 1922.)

FUNGI ASSOCIATED WITH "DIE BACK" IN STONE FRUIT TREES. I.[1]

By DOROTHY M. CAYLEY.

John Innes Horticultural Institution, Merton, Wimbledon.

(With Plates XI–XIV.)

INTRODUCTION.

The investigation as to the various causes of "die back" to which all stone fruit trees are so prone, was started with the study of a serious wilt and "die back" of Prince of Wales plum. This disease was first brought to the notice of the writer in 1919, by Mr Lobjoit, Controller of Horticulture. He described the disease as causing very serious damage, in Middlesex, to plum trees of the Prince of Wales variety, so much so, that there was considerable risk of this good market variety going out of cultivation. The investigation was then extended to all forms of "die back" in stone fruits, to see if "die back," in general, could be attributed to biological strains of one and the same fungus. Results, however, have shown that this is probably not the case. Four distinct fungi, easily distinguishable from one another, have been isolated from diseased tissues in "die backs" occurring on various kinds of stone fruits such as peach, apricot, nectarine, pear, different varieties of plums and also plum and apple stocks.

Three fungi only will be dealt with in this paper, namely *Cytospora* and *Eutypella*, on which a considerable amount of work has been done already by other investigators, and a new species of *Diaporthe*, which, so far, has not been recorded as being the cause of, or being associated with, "die back" in stone fruit trees in this country.

The causes of the so-called "die back" of fruit trees are still little understood, in spite of a considerable amount of literature which has accumulated on the subject. There appears to be a good deal of confusion owing to different investigators ascribing "die back" on different hosts to different organisms, and also to the fact that most, if not all of the

[1] A grant in aid of publication has been received for this communication.

fungi described, are alleged to have two distinct stages in their life-history, namely a pycnidial stage, in the form of *Phomopsis*, *Fusicoccum*, *Phoma* or *Cytospora*, and a perithecial or valsoid stage.

Massee in 1902 (35) investigated "die back" in young nursery fruit trees, more especially plum, apple, and pear, and attributed the disease to the fungus *Eutypella prunastri*. He also claimed to have brought about infection by applying spores of the conidial stage to lenticels in damp weather. There is, however, considerable doubt as to whether *E. prunastri* occurs on cultivated plum in this country, and if it does, it is far from common. For the last two years, during which a considerable number of specimens of "die backs" on various hosts have been examined, no *Eutypella* has been observed on cultivated plum, peach, apricot or apple, in spite of careful search.

True *E. prunastri*, however, does occur on wild sloe, wild plum, wild cherry, crataegus, etc. (46). *Eutypella*, as described by Massee, has one-celled ascospores, whereas all the perithecia found during the present investigation, with one single exception, contained bicellular ascospores. Since Massee's publication, it seems to have been generally accepted that "die back" is caused either by *E. prunastri* or by *Cytospora leucostoma* or *C. rubescens*. Both Cooke (5) and Saccardo give *C. rubescens* as the conidial stage of *Eutypella*.

Epidemics of "die back" attributed to *E. prunastri* have been reported from time to time. In 1902, in the *Woburn Report* (60), an interesting description is given of a serious outbreak of "die back" in a plantation of young plum trees on the estate, in which 41 per cent. of the trees, from four to nine years old, were killed in the course of three to four years. Different investigators attributed the disease to different causes, including *Eutypella*. Some of the symptoms described agree very closely with those observed by the present writer in cases of "die back" due to *Diaporthe*. This outbreak seems to have been successfully stopped by cutting away all diseased parts, and by removing all dead or dying trees. In 1910 (51) another serious outbreak of *E. prunastri* was reported as occurring in Kent, Worcestershire and Herefordshire, but no description of the fungus was given.

Besides *Cytospora* and *Eutypella*, other members of the genera *Phomopsis*, *Fusicoccum*, *Phoma*, etc. with or without a known ascigerous stage, have been recorded as causing disease on widely different host plants, some—more especially in the case of hard-wooded plants—associated with "die back," others as causing rots, etc. Further extensive investigation, together with cross inoculations, alone will show

whether the organisms occurring on closely allied hosts, the different stages of which have been described by different investigators under various names, may not be strains of the same fungus. It is not possible to give a complete list, but references to the more important and better known diseases caused by fungi belonging to, or closely allied to, the above genera, are given in the bibliography.

(1) CYTOSPORA.

After years of controversy (1, 14, 16, 17, 34, 55, 56), between pathologists in Germany, there appears to be no doubt that Aderhold (1) was able to prove by inoculation experiments that a *Cytospora*, with a valsoid stage, having gained access as a saprophyte on dead twigs, etc. can spread through the living tissues as a parasite and cause "die back" of cherry in Germany. Rolfs (48) in America also made successful cross inoculations with both pycnospores and ascospores of *Cytospora* on peach, plum, apricot, red cherry, and wild cherry.

On the other hand, Wormald (62), working on cherry "die back" in this country, failed to bring about the disease by inoculation, although he always found *C. leucostoma* associated with "die back" on cherry. He also failed to find mature perithecia.

It is difficult to say whether this strain of *C. leucostoma* is the same as the *Cytospora* with the valsoid stage occurring in Germany and America, more especially as perithecia of *C. leucostoma* have not been observed so far on stone fruits in this country.

Belgrave (4) has described *Cytospora* as occurring on Victoria, Prince of Wales and Pond's Seedling plums, but he did not make inoculations with pure cultures, and was not able to find mature ascospores. He found that "die back" was followed by the formation of pycnidia with red gelatinous tendrils of the genus *Cytospora*, "the members of which are recognised as conidial stages of various species of *Valsa*."

In the opinion of the writer, it is doubtful, judging from first-hand experience, and the failure of the above workers to bring about infection by inoculation, whether, with the exception of cherry and peach, the strain of *Cytospora* found in this country associated with "die back" in stone fruits, is really the primary cause of the disease. During the present investigation, the perithecial stage of this fungus has never been found, and in no instance have cultures of ascospores from valsoid perithecia on cultivated fruit trees, given rise to the conidial stage of *Cytospora*. Valsoid perithecia do occur in considerable numbers on branches suffering from "die back" on different hosts; but these perithecia, which have

always shown two-celled ascospores, belong to *Diaporthe*, a fungus entirely distinct from *Cytospora* or *Eutypella*, both of which have unicellular acospores.

A detailed description of *C. leucostoma* is to be found in Wormald's paper (62) on "The *Cytospora* disease of the Cherry," and this description agrees in every detail, as far as the morphology of the pycnidial stage of the fungus is concerned, with the *Cytospora* observed on peach and different varieties of plum during the present investigation.

In the case of plum trees, *Cytospora* is very frequently present on the dead parts of trees suffering from "die back," but it appears to be secondary, and the cause of the mischief can generally be traced to a region lower down, where the tissues are still alive, but more or less moribund. The bark in this region may or may not show definite signs of disease, but if portions are kept under moist conditions in a petri dish, the *Phomopsis* stage of a *Diaporthe* will develop in the course of a few days. A very striking instance of this occurred at Cookham Dean, in a young plantation of standard trees of the Prince of Wales variety of plum, a considerable number of which had been killed. *Cytospora* was found on portions of the dead branches sent in for examination. The nursery was then visited and the dead and dying trees carefully examined. The trees had been grafted at soil level, and the scion taken up to form the stem of the young standard trees, and examination at the base of the stem in the region of the graft showed typical *Diaporthe* lenticel-like pustules on nearly all the trees. Chips of bark from near the graft from a number of the trees on being kept a few days under moist conditions, developed the typical *Phomopsis* stage, and in a few instances the *valsoid* stage of *Diaporthe*.

The trees had evidently died from the graft upwards, as the stocks were alive and apparently healthy.

So far, inoculations on Prince of Wales plum with pure cultures of *Cytospora* isolated from the same variety of host, made by inserting spores under the bark of one year old shoots, have given negative results. The wounds remained healthy, showed no signs of infection and healed up quite as well as the controls. Two sets of inoculations were made, one at the end of March, 1920, and the other at the end of May in the same year. The trees have now been under observation for rather more than two years.

However, so little is known, as yet, as to the conditions conducive to infection by *Cytospora* and other members of this difficult group of Valsaceae, that these negative results must not be taken as conclusive.

There are grounds for believing that *Cytospora* may be weakly parasitic and infect tissues which are not quite normal owing to other causes, such as bad cultural conditions, insufficient moisture, excessive or indifferent pruning, bad drainage, etc. or it may gain access into the host as a saprophyte living for a time on dead twigs, spurs, etc. and from thence spread to the living tissues. This was proved to be the case by Aderhold with cherry "die back" (1) and Hubert with *C. chrysosperma* on certain forest, shade, and ornamental trees (30).

There may be and probably are many strains of *Cytospora*, some more virulent than others, some capable of forming perithecia, others not, and so on. So far, no attempt has been made to differentiate between strains of *Cytospora*. One point, however, has been observed by Belgrave, Aderhold, and the present writer, and that is, the variability of colour in the tendrils or globular exudations of *Cytospora*, from deep red to pale yellowish pink. This variation does not seem to depend, as do the size, shape and length of the tendrils, on the amount of external moisture present.

Peaches, grown out of doors, when attacked by *Cytospora*, show definite cankers, accompanied by exudation of gum on the older stem and branches (Pl. XI, fig. 2). On the younger growth the fungus girdles the shoot, resulting in rapid wilt and "die back" of the parts affected.

(2) *DIAPORTHE PERNICIOSA.*

This fungus has proved to be far more prevalent on portions of various stone fruits suffering from "die back" than any other fungus so far met with. It has been isolated from "die back" on peach, peach stock, apricot, pear, apple stock type II (Malling), wild sloe, and the following varieties of plum, Prince of Wales, Pershore, Victoria, Coe's Golden Drop, Goliath, Greengage, various seedlings from the cross New Orleans × Victoria, seedlings of Victoria selfed, and other unnamed varieties sent in from various sources. Specimens of the disease have been received from Wisbech, Cambridgeshire, Surrey, Middlesex, Devonshire, Berkshire, Kent, Anglesea; and "die backs" have been reported from other fruit-growing areas.

The disease attacks trees of all ages, but the damage is more noticeable on young trees, when the disease generally kills the whole tree very rapidly after the first symptoms have appeared. After the examination of a considerable amount of material, the conclusions arrived at are: that the development of the fungus in the host tissues is slow; that infection occurs a considerable time—in some cases probably years—before

any definite external symptoms can be seen; but that, once wilting has occurred, the affected areas die very rapidly, and the fungus completes its life-history as a saprophyte on the dead wood.

The first symptoms may be either rapid wilting and browning of the leaves during the growing season; or premature yellowing and fall of the leaves in autumn.

The bark at first looks healthy, and frequently no external symptoms can be seen before wilting sets in. In other cases the bark may take on a reddish tinge, or slightly sunken areas may extend longitudinally down the stem or branch. The smaller branches or stem instead of being cylindrical are inclined to be angular, or flattened, and this appearance becomes more accentuated as the disease progresses. This angular appearance is due to the uneven development of secondary thickening in the parts affected. The development of secondary thickening is arrested in the diseased areas, whereas normal growth continues in the parts of the periphery unaffected by the fungus. Pl. XI, fig. 5 shows a cross-section of a small branch, cut in December, in which the previous summer's secondary thickening has developed on one side only, and has been arrested round the rest of the stem. Infection in this case must have taken place during the previous winter or early spring.

Very frequently a considerable amount of callus is formed at the edges of the healthy tissue; this is most marked when the callus is formed on both sides of the sunken area, with the result that the bark is burst longitudinally down the stem, the wood laid bare, and the callus exposed, thus increasing the risk of secondary infection by other fungi and bacteria. Longitudinal splits of considerable length are of common occurrence on the branches of Prince of Wales plum and on the stems of young standard trees when grafted at soil level.

As the disease develops, transverse elongated excrescences form on the bark, which to the naked eye are very similar to, but much more numerous than, the undeveloped lenticels on healthy bark (Pl. XII, fig. 1). These excrescences are caused by the development of stromata of mycelial tissue immediately beneath or in between the external layers of cork. The bark is finally ruptured by the rapid growth of the stroma, and the spores are liberated from pycnidia embedded in the stroma, in the form of mucilaginous threads or tendrils, white or whitey buff in colour. These tendrils vary in length and thickness (Pl. XI, fig. 6). Under very moist conditions the spores may emerge as mucilaginous globules, which spread over the surface of the bark.

The spores are given off in damp weather. In dry weather the bark

does not slit open so readily and the pycnidia may be unable to liberate the spores for an indefinite period, as long as external conditions remain the same. Under dry conditions the mucilage of the extruded tendril also dries up, and the tendrils become hard and brittle, but never appear to pulverise. The mucilage can be easily re-dissolved in water. Hence the spores of this fungus, like those of *Cytospora*, are probably not wind borne, but carried on the feet of birds, visiting insects, and by wood-boring beetles, or washed down the stem, or splashed from the soil and from tree to tree during heavy squalls of rain, cf. (28).

The perithecia mostly occur in the same stroma as the pycnidia, either singly or in groups, but in some cases the perithecia lie apart from the pycnidia and their necks emerge through bark which has not been previously ruptured by a pycnidial sorus.

The fungus kills the cortical tissues, phloem, cambium, and medullary rays, and penetrates the wood, causing considerable discolouration of the xylem. If a diseased stem is cut across before it is quite dead and dry, discoloured areas can be seen in the internal tissues, and the darkened medullary rays can be traced with the naked eye. When the tissues are very dry the discolouration is not so apparent. This discolouration is not limited to any particular area, xylem of all ages can be affected. The whole of the internal wood may be affected, or the discolouration may be restricted to wedge-shaped areas or scattered blackened spots or lines in the interior of the stem (Pl. XI, fig. 5). These areas extend longitudinally for a considerable distance. There is always more or less xylem discolouration in stems attacked by *Diaporthe*, but this discolouration must not be confused with that due to pruning scars which have not completely callussed over. Different varieties of plums vary within wide limits as to their capacity for healing over pruning scars.

In the case of *Diaporthe*, black wavy strands of cells filled with dark mycelium can be traced down the stem.

As observed by Goethe in the case of *Nectria*, and Wormald (62) in *Cytospora*, the mycelium of *Diaporthe* passes vertically through the vascular system (Pl. XIII, fig. 14) and horizontally through the medullary rays (Pl. XIII, fig. 15). In the medullary rays the mycelium passes from one cell to another mostly through the pits (Pl. XIII, fig. 15). The medullary ray cells are filled with coarse brownish mycelium, which becomes very much attenuated when passing through the pits; but although mycelium, both coarse and fine, frequently occurs in considerable quantity in the lumen of the vessels, the latter are rarely completely blocked by the fungus itself. Some of the older vessels are blocked with tyloses and

yellow deposits, probably of the nature of wound gum, but the number of vessels affected is not large enough to account for the sudden wilt. The walls of the cells are discoloured, and show a certain amount of disorganisation; in the case of the vessels, the mycelium is able to penetrate the cell wall. The wilting and death of the shoot is due to the destruction of the phloem, cambium, and cortical tissues, and the disorganisation and blocking of the medullary rays, and not to the mechanical blocking of the vessels by the fungus itself.

The Fungus.

Diaporthe is an Ascomycete belonging to the Sphaeriaceae group of the Pyrenomycetes; family Valsaceae; with two distinct stages, the *Phomopsis* of pycnidial stage, and the *Valsa* of perithecial stage.

The bark of plum at an early stage consists of several layers of cork cells, and the mycelium sometimes penetrates between these layers, and forms knots of twisted hyphae, either between or immediately below these corky layers (Pl. XIII, fig. 10). These knots develop into the stromata in which the pycnidia develop. As growth proceeds a wavy line appears in the median region of the stroma. This line, the proliferous stratum of the pycnidium (Pl. XIII, fig. 10 *h*), consists of finer, more deeply staining hyphae than the surrounding coarse mycelium of the stroma. The upper and lower layers of the stroma split apart and the split forms the irregularly shaped chamber of the pycnidium, which is lined throughout with sporophores, bearing pycnospores. The number of chambers varies according to the size and shape of the stroma, but the dividing walls do not completely enclose each chamber, so that the pycnidium of *Diaporthe* can be distinguished from that of *Cytospora* which is much more definitely multilocellate.

Two distinct forms of pycnospores or conidia are found in one and the same pycnidium, as described by Diedicke and Grove (12, 18) in the genus *Phomopsis*, and by Harter and Field (25) in *Diaporthe batatis*, namely, long filiform hyalin spores (Pl. XIII, figs. 2, 7) definitely hooked at one end (rarely straight), thickening slightly in the middle region 25–28 $\mu \times 1\,\mu$, and called by Diedicke and Grove (12, 18) "b" spores, by Nietsche (43) stylospores, and by Marchal "basides" (38); and unicellular hyalin fusiform or elongate oval spores, 7–9 $\mu \times 2$–3 μ, with two large and several smaller oil drops, the "a" spores (Pl. XIII, fig. 1).

The filiform "b" spores are given off from short, subulate (Pl. XIII, fig. 6 *a*), and the "a" spores from long, simple, cylindrical, or slightly subulate sporophores (Pl. XIII, fig. 6). The filiform spores are always

produced first in the young pycnidium, to be followed later by the development of the "a" spores, hence the first tendrils consist mostly of "b" spores. Later, the two kinds of spores are found together in the same tendril, and finally, oval spores only are given off. Microtome sections have definitely shown that "a" and "b" spores can occur in the same pycnidium. The spores are given off in enormous numbers from the sporophores, and the cavity of the chamber is completely filled with abstricted spores before dehiscence occurs. It was not possible to ascertain whether each conidiophore is capable of abstricting more than one spore, or more than one kind of spore.

The ratio of length to breadth of the oval spores varies considerably according to age (Pl. XIII, fig. 1). The mature spores are wider and shorter owing to the development of two large oil drops which push out the sides and increase the width of the spore at the expense of the length. The filiform spores contain no oil drops.

The spore-tendril emerges either through a definite short-necked ostiolum at the apex of the pycnidium (Pl. XIII, fig. 11), or it may burst through the wall of the pycnidium. In the latter case the tendril is generally coarse and short as compared with the long fine tendrils, which may attain a length of 0·5 cm., given off from the ostiolum (Pl. XI, fig. 6). The tendrils, when first extruded, are whitey buff in colour, generally paler when they consist of filiform spores only; but later, when the tendrils have dried somewhat, they become yellowish in colour, and might be mistaken for the paler forms of *Cytospora* tendrils. It is difficult to say what function, if any, the filiform spores perform. Plate cultures of filiform spores gave no growth; numerous hanging drop cultures with different media were unsuccessful except in two instances. A preparation was made with acid pea-agar, 0·1 per cent. N. HCl, as the medium, and a tendril of filiform spores taken direct from the bark of Prince of Wales plum. On this medium, the filiform spores threw out lateral processes as in Pl. XIII, fig. 7, which had all the appearance of germ tubes, but unfortunately the cover slip was broken in an attempt to focus the culture under 1/12 oil immersion. Another attempt was made with the same medium; but, although spores in this case showed signs of germination, they did not advance so far as in the first instance.

There are differences of opinion as to whether these filiform bodies are really functionless spores, or merely some form of paraphyses (Reddick(45)). In stained preparations, no definite nucleus could be found in the "b" spores: the cell contents are more or less granular, and stain deeply. The absence of any definite nucleus rather points to their

being paraphyses—more especially as disintegrating "b" spores can be frequently seen in the older pycnidia.

The oval "a" spores, on the other hand, germinate quite freely on a considerable number of different media, giving rise to the typical *Phomopsis* stage with "a" and "b" spores.

The strain isolated from apricot has only been found in the *Phomopsis* stage, and differs somewhat as to its morphological characters from the strains on plum and peach. The "a" spores are rather more pointed and fusoid, $7{\cdot}5–9\,\mu \times 2{\cdot}5–3\,\mu$, and "b" spores are longer and arcuate, $40–50\,\mu \times 1$.

Perithecial Stage.

When the pycnidia have opened out and are quite empty, the perithecia begin to form in the deeper-seated layers of the stroma or in the cortical tissues, either below, or by the side of the pycnidia (Pl. XIII, fig. 12), and the valsoid black necks of the perithecia mostly emerge from the same lenticel-like slits from which the pycnospores have been liberated. The base of the pycnidium is pushed up by the developing perithecia, and in its turn is burst through by the growth of the necks of the perithecia. The walls of the perithecium consist of several closely packed layers of dark thick walled mycelium, enclosing several thin delicate hymenial layers. The perithecia contain numerous asci, $50–60\,\mu \times 7{\cdot}9\,\mu$ (Pl. XIII, fig. 8), with eight two-celled ascospores, $10–17\,\mu \times 2{\cdot}5–5\,\mu$ (Pl. XIII, fig. 3). The asci are furnished with two small lobes or pores at the apex of a short neck, which can be seen very readily when stained, *intra vitam*, with a saturated solution of erythrosin in glycerine. Each cell of the bicellular ascospore contains two large oil drops.

The necks of the perithecia are long, 1–3 mm., hairy and brownish in colour when young; black and glabrous when fully mature; bent in various directions and rather irregular in shape (Pl. XI, fig. 7), with a small pale brown area at the tip. They may occur singly, or, as is more generally the case, in groups or linear series from 3–24, according to the size of the stroma and the slit in the bark. The ascospores are liberated as whitey buff globules either through the ostiolum at the apex, or from any part of the neck. It requires moist conditions for the liberation of the ascospores from the neck of the perithecium. Thus it was found difficult to obtain pure cultures of ascospores from naturally dehiscing perithecia, owing to contamination by bacteria and various rapidly growing superficial fungi. Good cultures, however, can be easily obtained, by cutting away the diseased cortical tissues round a group of perithecia, and by

turning back the cut portion. The surrounding cortical tissues can then be carefully dissected away and the spherical bases of the perithecia exposed. The uninjured perithecia are then burst by slight pressure with a sterile needle and the ascospores are ejected as a mucilaginous globule, from which, with care, a fairly pure culture can be obtained. The ascospores appear to require a period of rest before germination. Ascospores obtained from old specimens which had been in the laboratory for many months germinated freely, whereas repeated attempts to germinate ascospores from freshly gathered material which had not completely dried out, failed.

Polymorphism of the organism on artificial media.

The forms of the stromata or pustules in which the pycnidia are developed vary considerably on artificial media. The more usual form of the pycnidium is more or less spherical, but aggregations of pycnidia may give rise to irregular masses, or at times the stroma may develop into a columnar or clavarieform structure (Pl. XI, fig. 4).

The same variation has been observed and fully described by the Marchals, and they point out that *Diaporthe perniciosa* shows "such a degree of polymorphism that one asks oneself if the types described under various names are not within the limits of the variability of this type."

Although not so marked, there is a certain amount of polymorphism in the fungus on the host plant, as to size and shape of stroma, number of pycnidial loculi, which vary with the size and shape of stroma; length of pycnidial and perithecial necks; variability in size and shape of pycnospores and ascospores according to age; presence or absence of "b" spores in the pycnidium, etc. It becomes more and more apparent that an adequate classification of this difficult group of fungi cannot be based on morphological characters and measurements of spores only.

PLATE CULTURES.

So far no medium has been found on which *Diaporthe* will go through both stages of its life-history satisfactorily. Platings of mass infections (*i.e.* numerous spores), and also single "a" pycnospore cultures, give rise very readily to the typical *Phomopsis* stage with both "a" and "b" spores on most of the media used; but, so far, perithecia have only developed from sowings of pycnospores in two cultures on sterile plum wood, and in one mono-pycnospore culture, isolated from a tendril with both "a" and "b" spores. Immature but normally developing perithecia were found in a sub-culture of the last-mentioned mono-pycnospore

culture on prune-juice-agar 69 days old. This culture had previously developed the typical *Phomopsis* stage with both kinds of pycnospores. Black stromatic patches were found embedded in the agar beneath these patches. Another sub-culture of the same mono-pycnospore strain grown on sterilised twig of plum developed mature perithecia with typical eight-spored asci and uniseptate spores. Inoculations with this strain, and yet another pycnospore culture from a different source, have brought about typical "die back" on the current year's growth of plum, and have developed perithecia quite freely on the host plant.

On the other hand, platings of mass infections of ascospores obtained by the method above mentioned, namely, that of bursting perithecia from the under side with a sterile needle, have given rather puzzling results. Out of 11 platings taken direct from perithecia, which showed no signs of contamination, six gave the typical *Phomopsis* stage with "a" and "b" spores, and five gave pycnidia with "a" spores only. The media used for these platings were prune-juice-oat-agar, and oat-agar, but the cultures showed quite definitely that the suppression or development of "b" spores does not depend on the medium. Of the six platings which gave "b" spores, three were on prune-juice-oat-agar and three on oat-agar, and the remaining five were also grown on both media. Single ascospore cultures, however, have, so far, only produced pycnidia very slowly and sparingly. The few pustules formed appeared typical, but did not dehisce readily, and in some instances it was found necessary to open the pustules with a sterile needle in order to examine the spores; "b" spores have never been found in the mono-ascospore cultures on any medium. The "a" spores in these cultures are rather undersized and the two oil drops very pronounced, presenting much the same appearance as the older spores from tendrils on the dryer portions of the bark on the host plant.

Mono-ascospore Cultures.

Some mono-ascospore strains produce no pycnidia, others only sparingly, and the general appearance of the cultures differs somewhat from the typical mono-pycnospore strains with "a" and "b" spores. The ascospores take nearly a week to germinate and the growth of the colony is somewhat slow. At first the surface mycelium is white, but later, on some media, such as oat-agar and oat-agar + 1 per cent. of glycerine, it becomes slightly tinged with pink. Blackened irregular patches of stromatic growth develop on the surface of the medium, forming a thin crust consisting of coarse dark mycelium with thick walls. The

perithecia mostly develop in the agar beneath these stromatic patches, and the necks protrude through the superficial crust. If there are pycnidia present, some perithecia may develop in the deeper seated portions of the pycnidial stroma.

The pycnospores in such cultures are generally shorter and wider than typical pycnospores and the oil drops very pronounced. No columnar development has occurred in mono-ascospore cultures.

At first it was thought that these differences indicated the presence of two distinct fungi in close association one with the other, but judging from the results as a whole the more probable explanation is that several biologic strains exist in this variable species.

In a recent publication Ém. and Él. Marchal(38) have described a winter storage rot of apples and pears caused by a new species of *Diaporthe* named by them *D. perniciosa*, with a pycnidial stage, *Fusicoccum malorum*, which appears to be of real importance to fruit culture in the vicinities of Gembloux, Huy, Brussels, etc. Besides causing rot of stored fruits, the fungus can attack unripe fruits of plum and peach; the fruits become completely covered with stromata, dry out and fall off. Cankers also occur on the stems and branches, more especially on apple and pear, more rarely on plum and cherry. Perithecia only appear when the fruits are completely mummified, and only "capricieusement" on the bark of affected trees. The *Diaporthe* causing "die back" of plum, etc. in this country agrees very closely as to its morphological characteristics with *D. perniciosa* with the exception of the dimensions of the asci. A specimen of "die back" on plum was forwarded to Marchal for comparison with his fungus, and he is of the opinion that the two organisms are the same, and that the slight differences observed are within the limits of the variability of this species.

As far as is known to the present writer, no species of *Diaporthe* has been recorded so far as causing "die back" of stone fruit trees in this country. Until this investigation is completed, however, it is thought advisable to adopt provisionally the name of *D. perniciosa* Marchal, *f. pruni*, for the forms on peach and plum, and *Phomopsis perniciosa f. armeniaca* for the form on apricot, which, as mentioned above, shows certain morphological differences.

CULTURES OF PYCNOSPORES OF *D. PERNICIOSA*.

Cultures from tendrils containing either "a" or "a" and "b" spores grow well on various artificial media, such as prune-juice-oat-agar (made with crushed oats); plum-shoot-decoction-oat-agar, plum-shoot-decoction-

agar, oat-agar, potato-agar, oat-agar + 1 per cent. glycerine, and also sterile shoots and blocks of plum wood; but less readily on potato or carrot. Large numbers of cultures isolated from different varieties of plum, apricot, peach, etc. kept under observation many months, have shown that the fungus will live a long time in pure culture.

Oat-agar. Mycelium white, felt-like, with greyish hairy pustules on the surface; submerged pustules show as black spots in the medium; medium not discoloured. In mono-pycnospore plate cultures mycelium in centre of colony raised and weft-like; pustular formation inclined to be zoned; pycnidia develop in large numbers with "a" and "b" spores; perithecia found only in one culture on this medium.

Plum-shoot-decoction-agar. Mycelium whitey-buff, fibrillose; pustules only slightly raised when single, but conglomerate pustules considerably raised above the surface of the medium, blackish, not so hairy as on oat-agar; medium discoloured reddish-brown; pycnidial development fair, not so good as on oat-agar; both kinds of spores. In mono-pycnospore cultures pycnidial formation zonal; no perithecia.

Plum-shoot-oat-agar. Mycelium white at first then turning buffish in colour, felt-like; pustules dark grey-green; colour of medium darkened. In mono-pycnospore colonies pycnidial development zonal; growth good; no perithecia.

Sterile wood. Growth sparse on heart wood; on younger wood mycelium whitish, spreading all over the wood; surface of wood blackened; pustules few, hairy, greyish-green, raised, some columnar; "a" and "b" spores; perithecia formed in two cultures.

Prune-juice-oat-agar. Mycelium loose at first, greyish; surface of medium frequently much creased; medium slightly discoloured; pustules raised, hairy, greyish-green in colour; good pycnidial development sometimes columnar; "a" and "b" spores; no perithecia.

Oat-agar + 1 per cent. glycerine. Mycelium at first white, turning pale greeny grey, rather loose; medium not discoloured; pustules raised but not columnar, hairy, greyish-green; good pycnidial development; no perithecia.

Potato. Growth poor and slow; pustules few, sometimes columnar; pycnidial development sparse.

Potato-agar. Mycelium loose, browny grey; pustules raised, hairy; medium slightly discoloured; good pycnidial development; "a" and "b" spores; no perithecia.

Sterile plum shoot. Mycelium loose, white at first, later tinged with buff; pustules hairy, raised, greyish-green; good pycnidial development; "a" and "b" spores; perithecia in one mono-pycnospore culture.

Carrot. Growth poor.

INOCULATION EXPERIMENTS.

Young Prince of Wales trees were inoculated in March and May of 1920 with various pure cultures of *Diaporthe*. A T-shaped incision, or a slit, was made in the bark as for budding, the bark raised and spores or mycelium inserted under the bark. The wound was then bound round with bast which had previously been boiled several times, and the whole covered with sheet gutta-percha, similarly treated. The hands of the operator were dipped in methylated spirit between each change of culture. The controls were treated in the same way.

The trees were inoculated on branches of the previous year's growth. So far, no typical wilt has resulted from these inoculations. The controls healed up quite healthily. The various cultures of *Diaporthe*, including the *Diaporthe* isolated from apricot, mostly showed more or less gumming and the wounds became sunken and unhealthy; inoculations with *Cytospora* isolated from Prince of Wales plum healed up quite as well as the controls. The trees have now been under observation for about 18 months.

On the other hand, the same cultures as those used for Prince of Wales plum on the young current year's growth of peach and nectarine in June, gave positive results in a comparatively short time.

(1) *Phomopsis* stage of *Diaporthe* isolated from bark of Prince of Wales on prune-juice-oat-agar 71 days old, produced rapid wilt on nectarine "Elruge" in 25 days (Pl. XIV, fig. 1 (2)); two inoculations, both positive.

(2) *Phomopsis* stage of *Diaporthe* isolated from bark of Coe's Golden Drop on oat-agar, 32 days old; rapid wilt on peach (var. unknown) in 25 days (Pl. XIV, fig. 1 (4)); three inoculations all positive.

(3) *Phomopsis* stage of *Diaporthe* isolated from bark of apricot on prune-juice-oat-agar, 21 days old; two inoculations with spores, on peach (var. unknown) positive in 64 days (Pl. XIV, fig. 2 (1)); and a third with mycelium as inoculum, positive after one year and 24 days.

(4) *Phomopsis* stage of *Diaporthe* isolated from bark of peach (var. unknown) on prune-juice-oat-agar, 44 days old, wilt in 17 days; two inoculations, both positive.

(5) *Phomopsis* stage of *Diaporthe* isolated from the bark of apricot on prune-juice-oat-agar, 52 days old; on young current year's growth of apricot, var. Breda; one inoculation positive after about one year.

(6) *Phomopsis* stage of *Diaporthe* isolated from the bark of Prince of Wales plum, on prune-juice-oat-agar, 73 days old; on young current year's growth of apricot, var. Kaisha; one inoculation positive after ten months.

(7) *Phomopsis* stage of mono-pycnospore culture of *Diaporthe* isolated from bark of Prince of Wales plum, on oat-agar, 18 days old (mycelium used as inoculum); on Jefferson plum; one inoculation positive after 61 days. (N.B. This mono-pycnospore culture produced perithecia on the host plant in three months.)

(8) Same culture as in (7) on young current year's growth on peach seedling (Royal George selfed); one inoculation positive; shoot dead after 11 months.

The above results show that the period which elapses between infection and the onset of the disease is very variable, and probably depends more upon the age and succulence of the parts infected, than the age of the cultures used.

Young peach shoots are more succulent than either plum or apricot. The above inoculations on the current season's growth of the various hosts were made during the months of June and July.

The organism was re-isolated from the tissues above and below the point of inoculation in at least one specimen of each of the above cases. These results prove quite definitely that all the above strains of *Diaporthe* are parasitic and cause "die back" on peach, nectarine, apricot and plum. The failure to bring about wilt on Prince of Wales plum is probably due to the inoculations having been made on older wood, and the organism has not yet permeated the tissues sufficiently to cause wilt.

Unripe apples of the varieties Lord Suffield, Peasgood Nonsuch and Bramley Seedling (Pl. XII, fig. 4) were inoculated through an incision in the skin, with mono-ascospore and mono-pycnospore cultures of *Diaporthe* isolated by the author from "die back" on Prince of Wales plum and Victoria seedling, and with a monospore culture of *D. perniciosa* forwarded by Prof. Marchal. Mycelium was used for the inocula in the case of Marchal's culture and the mono-ascospore cultures, and spores in the case of the pycnospore cultures. Decided patches of rot, identical in appearance, resulted in a few days from inoculations with plum ascospore and Marchal's cultures on Lord Suffield apples, and by plum pycnospore and Marchal's cultures on Peasgood Nonsuch. Bramley Seedling, a later apple, which was consequently still very hard and green, proved more resistant, and 14 days after inoculation only showed slight traces of rot.

Inoculations were also made on fruits of Victoria plum (Pl. XII, fig. 5) in various stages of ripeness from green fruits to those showing colour, with Marchal's culture and various other mono-ascospore and mono-pycnospore cultures. Marchal's culture proved to be the most virulent, giving rise in a few days to dark patches of rot $\frac{1}{2}$–$\frac{3}{4}$ inch in diameter, on

all the fruits inoculated. The patches of rot caused by the author's cultures appeared somewhat paler in colour. When pycnospores were used as inoculum the rot did not develop so rapidly, probably owing to the time required for the germination of the spores.

All the controls remained healthy.

The recent investigations of Wiltshire(59) as to the causes of "die back" have given interesting results. He describes the presence of bacterial masses in the diseased cortex together with fungal hyphae, the latter, in some instances developing cytospora-like fructifications, and suggests that there may be some relationship between the two organisms.

So far he has been unable to grow the bacteria on artificial media, and gives no description of the accompanying fungus. But it is quite possible that his work may throw light on the problem of the parasitism of *Cytospora*, and may show that concomitant infection with other organisms may be necessary in order to enable *Cytospora* to develop as a parasite in the host tissues.

The present writer has also observed rod-shaped bacteria in the disorganised cortical tissues attacked by *Diaporthe*; but, seeing that inoculations with *D. perniciosa* in pure culture have given definite results, more especially on peach, that they have caused considerable disturbance in one year old wood of plum, and also that they have set up rot in unripe apple and plum fruits, no concomitant organism appears to be necessary in order to bring about infection with *D. perniciosa*.

CONTROL MEASURES.

In spite of so little being known as yet as to the conditions which are conducive to infection of stone fruit trees by the fungi described in this paper, a few suggestions for control measures can be made.

Good cultural conditions and careful pruning are of prime importance. These fungi are all believed to be wound parasites and hence all cut surfaces should be treated with styptic or some antiseptic dressing. Very susceptible varieties, such as Prince of Wales plum, should be budded rather than grafted, and, if possible, well above soil level. In the case of standards the stock should be taken up to form the stem of the tree, and budded or grafted at the height required. The buds should not be taken from young, but from older trees, which have survived for a considerable number of years.

Care should be taken to examine the internal tissues of the shoots from which the buds are taken, and any shoot showing any traces of internal discolouration should be rejected.

When the bud has grown, the portion of the stock above the bud should be cut away and not left to act as a support for the growing shoot, as is so often the practice in nurseries; the cut surface should be pared smooth and treated with styptic. The same precautions hold good in the case of grafting, and the internal tissues of the stock also should be above suspicion.

Nothing is known as yet as to whether the variety of stock has any influence on the resistance or susceptibility of the scion. The stocks should be grafted young in order to avoid any great disparity in size between stock and scion, and to enable the wound to callus over completely.

The examination of a considerable number of young standard trees attacked by *D. perniciosa* has shown that in the majority of cases infection has occurred in the region of the graft, or a large pruning scar which has not healed over completely.

As regards the treatment of older trees, all diseased or dead parts should be carefully pruned back to healthy wood and the pruning scars treated as advised above. In damp weather the various fungi sporulate freely on dead or dying wood, hence all refuse and prunings should be burnt, and not left lying on the ground to infect the soil and the surrounding trees.

Implements used for cutting away diseased portions should be disinfected before use on healthy trees, more especially after a spell of wet weather. Saws, knives, the hands of the operator, etc. can be dipped in a 3 per cent. watery solution of lysol, but the lysol should on no account be used for treating pruning scars.

Specimens of mature pycnidia have been found on the host plants from January to September, and perithecia develop promiscuously throughout the year. The numbers of perithecia found are few as compared with the excessive development of pycnidia on the affected trees. This suggests that only certain strains of pycnospores are capable of forming perithecia and that the pycnidial stage is the parasitic stage. If spraying is resorted to, it should be done throughout the summer after rain; but this is hardly to be recommended on the score of expense.

In conclusion I wish to express my thanks to Mr Lobjoit, Controller of Horticulture, and Mr E. W. Lobjoit for bringing the disease to my notice and for giving me valuable information and every facility for collecting material and inspecting trees; to Mr A. D. Cotton and Mrs Alcock of the Pathological Laboratory of the Ministry of Agriculture for their help in supplying me with specimens and information as to the incidence

of "die backs" of fruit trees in various parts of the country; to Miss E. Gunnell, Horticultural Superintendent of Devon County Agricultural Committee, for supplying me with diseased specimens; to Prof. Marchal and Mr W. B. Grove for help in identifying the fungi described; to Mr Crane of this Institution; to Mr T. Richardson, County Inspector for Berks., and lastly to A. F. Emarton, laboratory assistant, for valuable help in making numerous microtome preparations and for taking the photographs for the Plates.

SUMMARY.

1. *Diaporthe perniciosa*, first isolated by Marchal from rots in stored fruits, has proved to be parasitic and one of the causes of "die back" in stone fruit trees.

2. The various strains of *D. perniciosa* isolated from "die back" on peach and different varieties of plums have proved to be parasitic, morphologically identical, and cause definite wilt and "die back" on peach, plum, apricot and nectarine. Cross inoculations of the same organisms on two year old wood of Prince of Wales plum have so far not brought about typical symptoms of "die back," but considerable disturbance, accompanied by gumming, has occurred at the points of inoculation. A strain obtained from apricot, which shows morphological differences, has also been proved to be capable of causing "die back" on peach and apricot. All the controls remained healthy.

3. The progress of *D. perniciosa* in the host tissue is slow; infection occurs a considerable time, in some cases years, before any external symptoms appear. The mycelium permeates the internal tissues more especially the medullary rays, cortical tissues, phloem cambium, and vessels.

4. *D. perniciosa* has a pycnidial or *Phomopsis* stage with "a" and "b" spores, and a perithecial or *Valsa* stage with numerous eight-spored asci. The pycnospores emerge as whitish tendrils or globules through numerous lenticel-like slits in the bark; the perithecial necks are long and protrude mostly through the same slits as the tendrils, and the ascospores are given off either through the ostiolum at the tip of the neck of the perithecium, or may burst through any part of the neck.

5. The external symptoms are sunken areas and longitudinal slits in the bark; elongated strands of numerous lenticel-like transverse slits through which tendrils and perithecial necks emerge; rapid wilt and browning of the trees in summer, or premature yellowing and fall of the leaves in the autumn.

6. The internal tissues are discoloured and secondary thickening arrested.

7. The pycnidial stage occurs on living or more or less moribund bark, the perithecial stage on the parts which are dead.

8. *D. perniciosa* shows a certain degree of polymorphism on the host plant; on artificial media this polymorphism is much more marked.

9. The *Phomopsis* stage of *D. perniciosa* grows and sporulates well on artificial media, but perithecia develop sparingly in cultures from pycnospores.

10. Cultures of mass infections of ascospores of *D. perniciosa* have given rise in some cases to the *Phomopsis* stage with "a" and "b" spores; in others no "b" spores could be found. Mono-ascospore cultures develop the pycnidial stage very sparingly, and no "b" spores have been observed in any of the cultures. Large numbers of perithecia develop in mono-ascospore cultures on various media.

BIBLIOGRAPHY.

(1) Aderhold, R. (1903). Über das Kirschbaumsterben am Rhein, seine Ursachen und seine Bekämpfung. *Arb. d. Biol. Abt. f. Land- und Forst-Wirthschaft am. kais. Gesundheitsamt*, Bd III, Heft 4.

(2) Appel, O. (1904). *Naturw. Zeitschr. f. Land- und Forst-Wirtschaft*, p. 313.

(3) Bailhache, C. H. (1920). *The Fruit Grower*, Nov. 25th.

(4) Belgrave, W. N. C. (1915). On Diseases of Plum Trees caused by some species of *Cytospora*. *Ann. Appl. Biol.* II, Nos. 2 and 3, July.

(5) Cooke, M. (1901–1902). *Journ. Roy. Hort. Soc.* XXVI.

(6) —— (1902). *Ibid.* XXVII.

(7) —— . *Fungoid Pests of Cultivated Plants.*

(8) Clinton, G. P. (1911–1912). Chestnut bark disease. *Conn. Agri. Exp. Stat. Report* (with good bibliography).

(9) Cotton, A. D. (1902). *Journ. Roy. Hort. Soc.* XXVII, 936.

(10) Darnell-Smith, G. P. (1918). An account of some observations upon the life history of *Phoma citricarpa*. *Proc. Linnean Soc. N.S. Wales*, 43.

(11) Delacroix, G. (1905). Sur une Maladie des Armandiers de Provence. *Bul. Trimestre Soc. Myc. de France*, XXI.

(12) Diedicke, H. (1911). Die Gattung *Phomopsis* und *Plenodesmus*. Preuss. *Ann. Myc.* IX.

(13) Dowson, W. J. (1913). On a Disease of Greengage Trees caused by *Dermatella prunastri*. *New Phytologist*, XII, No. 6.

(14) Ewert, R. (1908). Ist das Rheinische Kirschsterben auf eine Pilzkrankheit zurück zu führen. *Proskauer Obstbauzeitung*, Jahrg. 13.

(15) Farlow, W. G. (1912). The Fungus of the Chestnut Blight. *Science*, N.S., May.

(16) Frank, A. B. (1899). *Deutsche Landw. Presse*, No. 83.

(17) Goethe, R. (1899). Das Absterben der Kirschenbaüme in den Kreisen St Goar, St Goarhausen und Unterlahn. *Ibid.* No. 99.

(18) Grove, W. B. (1917). The British Species of *Phomopsis*. *Kew Bull.* No. 2.

(19) —— (1919). Species placed by Saccardo in the genus *Phoma*. *Ibid.* No. 4.

(20) HAHN, G. G., HARTLEY, C. and PIERCE, R. G. (1917). A nursery blight of cedars. *Journ. Agric. Res.* X.

(21) —— —— —— (1920). *Phomopsis Juniperovora. Phytopath.* X, No. 4.

(22) HARTER, L. L. (1913). Foot rot of Sweet Potato. *Journ. Agric. Res.* I, No. 3.

(23) —— (1914). Fruit rot, leaf spot and stem blight of Egg Plant, caused by *Phomopsis vexans. Ibid.* II, No. 5.

(24) HARTER, L. L. and FIELD, E. C. (1912). *Diaporthe*, the ascogenous form of Sweet Potato Dry Rot. *Phytopath.* II, No. 3.

(25) —— —— (1913). Dry Rot of Sweet Potatoes caused by *Diaporthe batatis. U.S. Dep. Agric. Bur. Pl. Ind., Bull.* 281.

(26) —— (1917). Pod blight of Lima Bean caused by *Diaporthe phaseolorum. Journ. Agric. Res.* XI, No. 10.

(27) HEALD, F. D. and GARDNER, M. W. (1913). The relative prevalence of pycnospores and ascospores of the Chestnut blight fungus during the winter. *Phytopath.* III, No. 6.

(28) HEALD, F. D. and STUDHALTER, R. A. (1914). Birds as carriers of the Chestnut Blight Fungus. *Journ. Agric. Res.* II, No. 6.

(29) HOEHNEL, VON (1906). *Fragmente zur Mykologie*, No. 87.

(30) HUBERT, E. E. (1920). Observations on *Cytospora chrysosperma* in the Northwest. *Phytopath.* X, No. 10.

(31) KEEPER, W. E. (1914). Pathological Histology of the *Endothia* canker of Chestnut. *Ibid.* IV, No. 3.

(32) LAUBERT, R. (1904). Zur Morphologie einer neuer *Cytospora. Cent. f. Bakt.* II, Bd. XII.

(33) LEWIS, C. E. (1909). Apple diseases caused by *Coryneum follicolum* and *Phoma mali. Maine Agric. Exp. Stat. Bull.* 170.

(34) LÜSTNER, G. (1907). Untersuchungen über die Ursache des Rheinischen Kirschbaumsterbens. *Ber. d. Königl. Lehranstalt für Wein-, Obst-, und Gartenbau zu Geisenheim am Rh.*

(35) MASSEE, G. (1902). Disease of Nursery Stock. *Gard. Chron.* Sep. 27th.

(36) —— (1902). *Journ. Bd. Agric.* IX.

(37) —— (1901–1902). *Journ. Roy. Hort. Soc.* XXVI.

(38) MARCHAL, ÉL ET ÉM. (1921). Contribution à l'étude des Champignons Fructicoles de Belgique. *Bull. d. l. Soc. Roy. de Bot. de Belgique*, T. LIV. N.S. IV.

(39) MURRILL, W. A. (1906). A serious Chestnut disease. *Journ. N.Y. Bot. Gardens*, No. 7.

(40) —— (1906). Further remarks on a serious chestnut disease. *Ibid.* No. 7.

(41) —— . *Torreya*, V, 6, No. 9.

(42) —— . *Ibid.* V, 8, No. 5.

(43) NIETSCHE, T. *Pyrenomycetes Germanica.* Valsaceae.

(44) POTEBNIA, A. (1907). *Fusicoccum pruni. Ann. Myc.* V.

(45) REDDICK, D. (1909). Necrosis of grape vine. *Conn. Univ. Agric. Exp. Stat. Dep. Pl. Path. Bull.* 263.

(46) REHM, H. (1910). *Eutypella prunastri*, f. Crataegi. *Ann. Myc.* VIII.

(47) ROBERTS, J. W. (1913). Rough bark disease of Yellow Newtown Apple. *U.S. Dep. Agric. Bur. Pl. Ind., Bull.* 280.

(48) ROLFS, F. M. (1910). Winter killing of twigs, cankers and sun-scald of peaches. *Missouri St. Fruit Exp. Stat., Bull.* 17.

(49) RUE, C. DE LA. (1920). Isolating single spores. *Bot. Gaz.* LXX, No. 4, Oct.

(50) RUHLAND, W. (1904). Ein neuer, verderblicher Schädling der Eiche. *Cent. f. Bakt.* Abt. 2, Bd. XII.

(51) SALMON, E. S. (1910). *Journ. S.E. Agric. Coll. Kent*, No. 19.

(52) Shear, C. L. (1917). End rot of Cranberries. *Fusicoccum putrefaciens. Journ. Agric. Res.* xi, No. 2.

(53) —— (1911). The ascogenous form of fungus causing dead arm of Grape. *Phytopath.* i, Nos. 3 and 4.

(54) Shear, C. L. and Wood, A. K. Studies of fungus parasites belonging to the genus *Glomerella. U.S. Dep. Agric. Bur. Pl. Ind., Bull.* 252.

(55) Sorauer, P. (1900). Das Kirschbaumsterben am Rh. *Deutsch. Landw. Presse*, No. 18.

(56) Wehmer, C. (1898). Zum Kirschbaumsterben am Rh. *Ibid.* No. 96.

(57) —— (1894). Über das massenhafte Vorkommen eines Kernpilzes auf den Alleebäumen etc. *Jahresber. d. Naturhis. Gesell.* Hanover.

(58) Wilson, M. (1919–1920). A new species of *Phomopsis* parasitic on the Douglas Fir. *Trans. and Proc. Bot. Soc. Edinburgh*, xxviii, Pt I.

(59) Wiltshire, S. P. (1920). A bacterial infection of plum trees. *The Annual Report, Long Ashton Exp. Stat. Bristol.*

(60) Woburn (1900). *Exp. Fruit Farm, Rept.* ii.

(61) Wolf, F. A. and Stanford, E. E. (1918). A *Macrophoma* disease of Figs. *Phytopath.* viii, No. 1.

(62) Wormald, H. (1912). *Cytospora* Disease of Cherry. *Journ. S.E. Agric. Coll. Wye, Kent*, No. 21.

EXPLANATION OF PLATES XI—XIV

PLATE XI.

Fig. 1. (1) Healthy stem. Prince of Wales plum. $\times\frac{3}{4}$. (2) *Phomopsis* stage of *Diaporthe perniciosa* showing tendrils of spores on Prince of Wales plum. (3) *Phomopsis* stage of *D. perniciosa* on stem of standard of seedling of Victoria plum.

Fig. 2. Canker on stem of peach seedling due to *Cytospora*, probably *C. leucostoma* var. *cincta.*

Fig. 3. Branch of seedling from Victoria plum, attacked by *D. perniciosa*, showing discolouration of internal tissues in (*b*) and (*c*); (*a*) healthy branch.

Fig. 4. Slope culture of *Phomopsis* stage of *D. perniciosa* isolated from peach, on potato + 1 % glycerine; 143 days old, showing columnar development of pycnidia. $\times\frac{3}{5}$.

Fig. 5. Sections of diseased branches of Prince of Wales plum attacked by *D. perniciosa*, showing arrested secondary thickening and discolouration of internal tissues.

Fig. 6. Tendrils of *Phomopsis* stage of *D. perniciosa* showing coarse and fine tendrils. $\times 6$.

Fig. 7. Perithecia of *D. perniciosa* on bark of Pershore plum. $\times 3$.

PLATE XII.

Fig. 1. Young tree of Prince of Wales plum attacked by *D. perniciosa* in the region of the graft. Pustules showing on the bark in the diseased area d–d_1.

Fig. 2. The reverse side of the same tree showing incomplete healing over of the graft wound.

Fig. 3. (*a*) Pycnospore culture of *D. perniciosa* isolated from peach on prune-juice-oat-agar, 24 days old. (*b*) Pycnospore culture of *D. perniciosa* isolated from Prince of Wales plum on prune-juice-oat-agar, 44 days old.

Fig. 4. Inoculations of apple fruits with *D. perniciosa* from various sources. (1) Peasgood Nonsuch; control. (2) Peasgood Nonsuch inoculated with mono-pycnospore culture from bark of Prince of Wales plum. (3) Lord Suffield inoculated with mono-ascospore culture isolated from bark of Prince of Wales plum. (4) Bramley Seedling inoculated with same culture as (2). (5) Bramley Seedling control. In (2) and (4) pycnospores were used as inoculum, in (3) mycelium.

Fig. 1

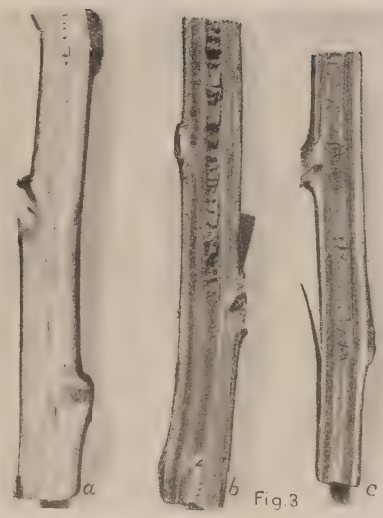


Fig. 3

Fig. 4

Fig. 2

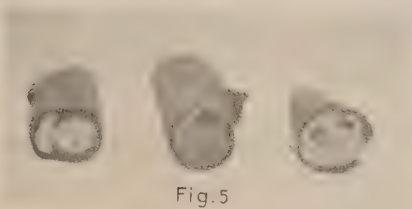


Fig. 5

Fig. 8

Fig. 7

Fig. 1

Fig. 2

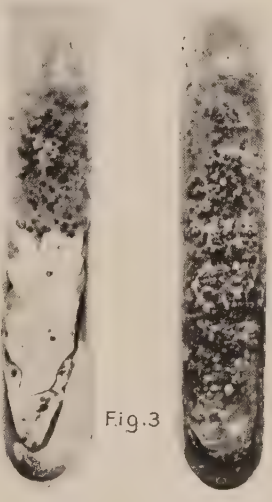


Fig. 3

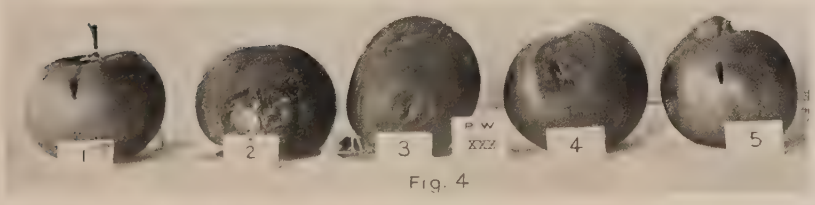
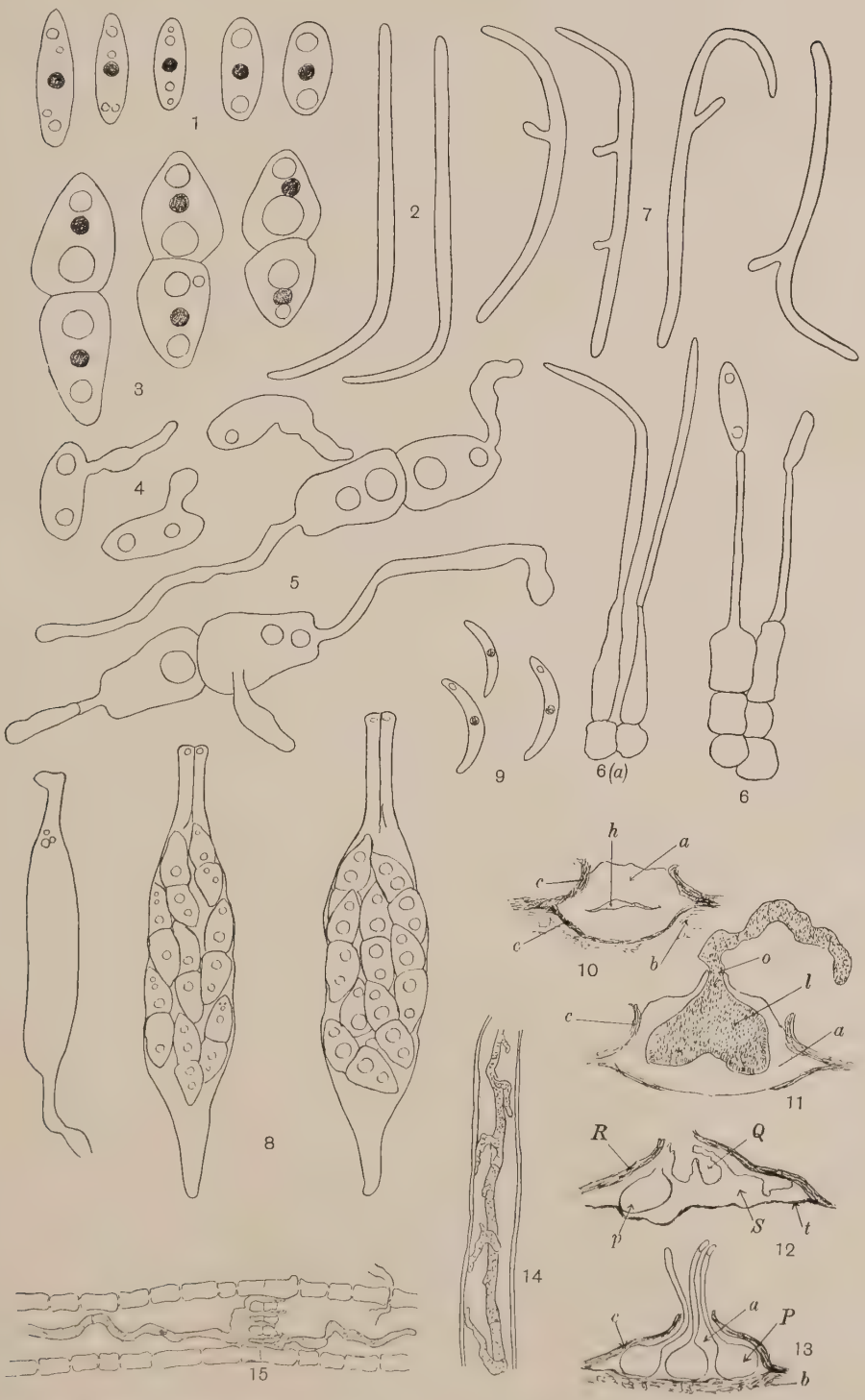


Fig. 4

Fig. 5

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)



CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

Fig. 5. Inoculation of Victoria plum fruits of varying degrees of ripeness, with *D. perniciosa* from various sources. (1) Control; ripe plum. (2) Ripe plum inoculated with multi-sporous pycnospore culture isolated from bark of Victoria plum. (3) Unripe plum inoculated with mono-pycnospore culture isolated from bark of Victoria plum. (4) Unripe plum inoculated with mono-ascospore culture isolated from bark of Prince of Wales plum. (5) Unripe plum inoculated with Marchal's mono-spore culture. In (3) pycnospores used as inoculum, in (2), (4), (5), mycelium.

PLATE XIII.

Fig. 1. "a" pycnospores of different ages. *D. perniciosa.* ×2000.
Fig. 2. "b" pycnospores. *D. perniciosa.* ×2000.
Fig. 3. Ascospores. *D. perniciosa.* ×2000.
Fig. 4. Germinating "a" pycnospores. *D. perniciosa.* ×2000.
Fig. 5. Germinating ascospores. *D. perniciosa.* ×2000.
Fig. 6. Sporophores and young "a" pycnospores. *D. perniciosa.* ×2000.
Fig. 6 *a*. Sporophores and young "b" pycnospores. *D. perniciosa.* ×2000.
Fig. 7. Germinating (?) "b" pycnospores. *D. perniciosa.* ×2000.
Fig. 8. Asci of different ages. *D. perniciosa.* ×1000.
Fig. 9. Pycnospores of *Cytospora.* ×2000.
Figs. 10, 11. Development of pycnidia in the sorus, *D. perniciosa.* ×20. (*a*) mycelial tissue of the sorus; (*b*) disorganised tissues of the cortex of the host; (*c*) corky layers of the bark; (*h*) proliferous layer of the young pycnidium; (*l*) loculus of the pycnidium; (*o*) ostiolum.
Fig. 12. Stroma of *D. perniciosa.* ×20. (*p*) young perithecium; (*Q*) disorganised chamber of empty pycnidium; (*R*) cork; (*S*) mycelial tissue of stroma; (*t*) black delimiting line of the stroma.
Fig. 13. Group of perithecia of *D. perniciosa*: (same figuring as in Figs. 10, 11).
Fig. 14. Mycelium of *D. perniciosa* passing vertically up a fibre.
Fig. 15. Mycelium of *D. perniciosa* passing horizontally through the pits of a medullary ray cell.

PLATE XIV.

Fig. 1. Inoculation on peach and nectarine. (1) Control (attacked by aphis). (2) Nectarine "Elruge" inoculated under the bark with pycnospores of *D. perniciosa* isolated from Prince of Wales plum; 26 days after inoculation. (3) Same as (2). (4) Peach (var. unknown) inoculated with pycnospores of *D. perniciosa* isolated from Coe's Golden Drop plum.
Fig. 2. (1) Peach (var. unknown) inoculated with pycnospores of *D. perniciosa*, isolated from apricot; 64 days after inoculation. (2) Control. × = point of inoculation.

(*Received November 7th*, 1922.)

ON A NEW SPECIES OF MONIEZIA FROM THE SHEEP, *OVIS ARIES*

By J. R. W. JENKINS, B.Sc.

Research Student in the Department of Zoology, University College of Wales, Aberystwyth.

(With Plate XV and 2 Text-figures.)

The present paper represents a part of certain work which I have been carrying out upon tape worms of the genus Moniezia, with the aid of a special research grant from the Ministry of Agriculture and Fisheries, and a supplementary research exhibition from the Honble. Samuel Vestey, to both of whom I would like to take this opportunity of tendering my thanks.

The material was collected, together with other species of Moniezia, from the small intestines of sheep killed at the Municipal Slaughterhouse, Aberystwyth.

General Appearance.

When fresh, the strobilia is extremely translucent, the anterior portion is clear and colourless, the posterior portion, due to the egg content of the gravid uteri, is dotted with yellow. The line of the ventral excretory canals is easily visible to the naked eye, as are the genital papillae.

Scolex.

The scolex is small, pear-shaped, and almost square *en face*. It varies in length from ·625 mm. to 1·25 mm., and in breadth from ·496 mm. to ·750 mm. Its distinguishing feature is, however, that in any individual scolex, the length always exceeds the breadth. The scolex tapers gradually towards the neck, from which it is not clearly separated. The suckers are situated anteriorly at the four corners. They are only slightly raised from the main surface level of the scolex, and are not distinctly separated from it. The average width of the suckers is ·250 mm. The sucker openings are oval in shape, and inclined diagonally forwards; their average breadth is ·04 mm., and depth ·06 mm. The sucker wall is exceedingly muscular and distinctly separated from the undifferentiated scolex parenchyma. It consists of a ground tissue of parenchyma com-

posed of well defined cells, and bounded by a strongly developed layer of circular muscle, and an outer, less well developed layer of longitudinal muscle. The ground tissue is very thickly interspersed with longitudinal, transverse and oblique muscles.

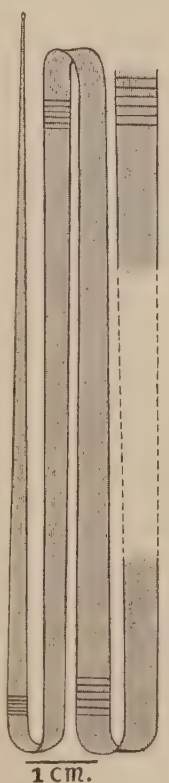
Neck.

The neck is long and slender, not distinctly separated from the scolex. It varies in length from 2·38 mm. to 4·63 mm., and in breadth from ·214 mm. to ·496 mm.

Strobila.

In common with those of the other members of the genus, the segments are always much broader than long, the terminal segments having a tendency to become longer and narrower. The strobila rapidly increases in width. At 10 cms. from the scolex, the average width of the segments is 2·75 mm., and their average length ·458 mm.; at this point there is no visible sign of reproductive organs. At 20 cms. from the scolex, the segments average 3·75 mm. in width, and ·81 mm. in length; the genital anlage is visible, and has the pistol shape, characteristic of the genus; the genital pores have not pierced the edge of the segment; the testes are visible. At 30 cms. from the scolex, the average breadth of the segments is 4·66 mm., and their average length 1·0 mm.; the reproductive organs are now clearly visible, and the genital pores have opened to the exterior, the genital papillae being clear and distinct to the naked eye.

The longest specimen obtained measured 185 cms. in length, the end segment, some segments evidently having become detached, being 9 mm. wide. The greatest width of segments observed was 12 mm. and greatest length 3 mm.



Text-fig. 1. Strobila of *M. translucida*. To show the rate of increase in the width of the strobila and in the length of the segments.

Histology.

The general histology of the species is typical of the genus, and calls for little comment. The muscular system is not well developed, particularly the circular muscle layer, whilst the constituent longitudinal muscle fibres in the older segments lose much of the

aggregation into little bundles which is typical of the younger segments. The external cuticular layer is thin.

Interproglottidal Glands.

The interproglottidal glands are linear. They are short, but well defined, varying in length from ·9 mm. to 1·6 mm. The length of the glands depends to some extent upon the width of the segments, but not wholly so. Thus, glands 1 mm. long have been observed in segments 3·6 mm. wide, and in segments 8·8 mm. wide, whilst in other segments 8 mm. wide, glands 1·6 mm. long have been observed.

Excretory System.

In the strobila the lumen of the dorsal canal is small, varying in diameter from ·004 mm. to ·008 mm. Its course is very irregular, it usually lies on the median side of the ventral canal, and slightly dorsal to it. It may, however, lie in the same dorso-ventral line as the ventral canal, or may even lie ventral to the centre point of the latter. Except in the scolex, the dorsal canal has not been observed to lie external to the ventral canal.

The cuticular lining of the dorsal canal is much thicker than that of the ventral canal, and contains darkly staining bodies resembling very small nuclei. The dorsal canal is surrounded by a well defined layer of round or oval cells, considerably larger than those of the surrounding parenchyma, and possessing large and well defined nuclei. The layer is usually one cell thick, sometimes two.

The lumen of the ventral canal varies in diameter from ·04 mm. to ·06 mm. At the posterior end of each segment, the ventral canals are connected by a transverse commissure of ·02 mm. diameter. The cuticular lining of the ventral canal is thin. The canal is surrounded by an ill-defined layer of flattened ovoid cells, with fairly well-defined nuclei.

In the unsegmented neck region, the dorsal and ventral canals are more nearly equal in diameter, ·008 mm. and ·012 mm. respectively. The dorsal canals lie slightly internal to the ventral canals, which are not connected by transverse commissures in this region.

In the scolex the canals are of the same dimensions as in the neck. At a point approximately in a line with the centre of the suckers, the ventral canals are connected by a transverse commissure; ·08 mm. anteriorly, the dorsal canal of each side unites with its ventral canal and ·02 mm. anterior to this union, the joint canals unite in the middle line.

In many instances it was noticed that the ventral canals gave off large longitudinal branches which ran through three or four segments and then joined up again with the parent canal, whilst in one scolex, the dorsal and ventral canals of one side only were united by a short commissure. The number and topography of the excretory canals in this species, therefore, would appear to be liable to slight variation.

In the original terminal segment of the strobila, the dorsal canal of each side unites with its ventral canal and the two joint canals open by a common pore in the mid-ventral line.

Nervous System.

Identification of the nervous system presented difficulties, no entirely satisfactory method of differential staining having been discovered. It has been possible to determine, however, that the nervous system is of the usual type. The cephalic ganglion is composed of numbers of transverse fibres massed together to form a nerve bundle, which runs across the scolex, passing between the dorsal and ventral excretory canals, immediately posterior to the fusion of the dorsal and ventral canals of each side as described above. The cephalic ganglion gives off nerve fibres to the four suckers, and a pair of main lateral longitudinal nerves. These latter run, one on each side, the whole length of the strobila, external to the excretory canals, crossing the genital ducts ventrally.

Reproductive System.

The genital papillae are easily visible to the naked eye, they are usually situated in the anterior third, and invariably in the anterior half of the lateral borders of the segment.

Cirrus and vagina open into the common genital papilla, on the right side of the segment the cirrus being dorsal to the vagina, whilst on the left-hand side, the cirrus is ventral to the vagina.

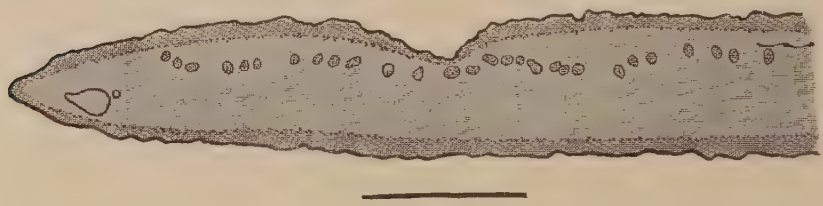
The antero-posterior relations of cirrus and vagina are not quite constant; on the right side of the segment the cirrus is usually anterior to the vagina, and on the left side, posterior to it. This condition occurs in about 75 per cent. of the segments. In the remaining 25 per cent. of the segments, cirrus and vagina of either side may lie in the same lateral plane, or, the vagina of the right side may be anterior, whilst the vagina of the left side may be posterior. The genital canals invariably cross both excretory canals and longitudinal nerve, dorsally.

Male Organs.

The cirrus pouch runs straight inward towards the median line, it averages ·12 mm. long and ·06 mm. broad. The expanded cirrus is ·14 mm. long, convolutions of the cirrus in the cirrus sac being uncommon.

The lumen of the cirrus at its lateral end averages ·004 mm. in diameter, widening gradually to ·02 mm. in diameter at its median end, where it constricts suddenly to ·004 mm. in diameter to leave the cirrus pouch. Immediately after leaving the pouch it dilates to ·04 mm. diameter to form the vas deferens, which, on the left side of the segment, at once crosses to the dorsal side of the vagina. The vas deferens performs numerous convolutions, whilst running inwards and downwards, crossing the ovary dorsally.

The testes are uniformly distributed throughout the median field in both young and old segments, and are confined to the posterior portion



·5 mm.

Text-fig. 2. T.S. of left half of a proglottid to show the distribution of the testes.

of the segment, not being found anterior to a plane passing through the anterior border of the shell gland. They are thus arranged in a long, narrow, rectangle. As regards their dorso-ventral distribution, the testes are invariably confined to a single dorsal layer. No testes were found in the lateral fields, that is, external to the excretory canals. In a mature segment, about 300 testes are visible.

The cirrus pouch is a very muscular organ, consisting of the following tissue layers in order from within outwards:

1. *Lumen.* The ciliary projections into the lumen described in other species of Moniezia have not been observed.
2. *Non-cellular layer.* This layer possesses well-defined nuclei.
3. *Circular muscle layer.*
4. *Longitudinal muscle layer.*
5. *Parenchyma.* The cells of which are large, round or oval in shape, and possess well defined nuclei.

6. *Circular muscle layer.* With large, deeply staining nuclei.

7. *Longitudinal muscle layer.*

The wall of the vas deferens consists of a thin basal membrane, which is invariably surrounded externally by a well defined layer of prostatic cells with large nuclei. The layer is one or two cells thick, the constituent cells being very similar in appearance to those of the shell gland.

The testes are round or oval bodies varying in diameter from ·075 mm. to ·1 mm. They are tightly packed with small, darkly staining nuclei. Cavities in the testes, in which sperms may be observed, are frequent.

Female Organs.

The vagina runs inwards from the genital papilla for ·3 mm. with a diameter of ·016 mm. It then dilates to form a short bulb, ·04 mm. long and ·02 mm. broad, which is separated by a constriction, ·02 mm. long and ·004 mm. in diameter, from the receptaculum seminis.

The latter is a large, thin-walled sac, ·5 mm. long and ·1 mm. in diameter, which runs inwards and slightly posteriorly, towards the shell gland. A short distance from the median extremity of the receptaculum seminis, and on its ventral side, a large duct, ·016 mm. in diameter, takes its origin. This duct immediately constricts to a diameter of ·008 mm., the constriction being followed by a dilated portion ·02 mm. in diameter. This duct is exceedingly muscular, its wall consisting of diagonal muscle fibres with well defined nuclei, surrounded by an outer layer of longitudinal muscle fibres. At the dilated portion referred to above, the duct divides into a ventral branch, the oviduct, and a dorsal branch leading to the shell gland. This point of bifurcation is ·016 mm. from the point of origin of the duct.

The oviduct is ·008 mm. in diameter. It consists of a basement membrane, and an internal epithelium, the constituent cells of which are large, and possess well defined nuclei. It follows a curved course, the convexity being ventral, and runs inwards anteriorly and ventrally, to enter the central portion of the ovary, from which it is separated by a constricted portion ·004 mm. in diameter, and a short bulb ·006 mm. in diameter. Both constriction and bulb have very muscular walls.

The shell gland duct is similar to the oviduct in structure, it is ·008 mm. in diameter. It runs dorsally, slightly inwards, and towards the posterior border of the segment, to enter the shell gland, in the centre of which it dilates to ·02 mm. From this dilation, two ducts take their origin, the vitelline duct, and the uterine duct.

The vitelline duct is ·004 mm. in diameter. It arises from the ventral portion of the dilation, runs a short distance ventrally, and then turns abruptly posteriorly to enter the yolk gland, where it dilates suddenly to a diameter of ·02 mm. It performs no convolutions.

The uterine duct arises dorsally from the median end of the dilation in the centre of the shell gland. It is much coiled, both inside and outside the latter. It runs dorsally for a short distance, then curves anteriorly and runs ventrally, to open into the uterus almost in the median line.

The vagina wall is similar in structure to the cirrus wall, the muscle layers, however, being in the reverse order. It consists of the following layers:

1. *Lumen.*
2. *Homogeneous layer*, possessing well defined nuclei.
3. *Longitudinal muscle.* Better developed at the median end than at the lateral end.
4. *Circular muscle.* Better developed at the lateral end than at the median end.
5. *Cellular layer* of large rounded cells with well defined nuclei.

The wall of the receptaculum seminis consists of a basement membrane enclosing a homogeneous layer with well defined nuclei.

The ovary is renal in form, its two extremities enclosing the shell gland and vitelline gland. It occupies a ventral position. Its average lateral dimension is 1 mm. and its diameter ·20 mm., both these dimensions varying considerably according to the degree of contraction of the segment. The cortical portion consists of a number of long, slender tubes, densely packed with large darkly staining nuclei, and radiating in a fan-like manner from the medullary portion, from which the oviduct takes its origin.

The shell gland is a compact organ, circular or oval in shape, lying in the median plane between the ovary and the vitelline gland. Its diameter varies from ·09 mm. to ·16 mm. It is composed of well defined or oval cells, tightly packed together and possessing large and well defined nuclei.

The yolk gland lies on the posterior side of the ovary and the shell gland, and slightly ventral to the latter. It is composed of a number of short, wide tubes, radiating from the central cavity from which the vitelline duct takes its origin. It is packed with darkly staining nuclei rather smaller than those of the ovary. The gland is oval in shape, its lateral diameter measures ·24 mm. and its transverse diameter varies from ·16 mm. to ·10 mm.

The uterus is a most complicated, much branched organ, the branches ramify throughout the entire segment, crossing the excretory canals dorsally to enter the lateral fields. The uterus wall is composed of a basement membrane.

Ova.

The eggs are more or less globular, tending to become cuboid on pressure. They vary in diameter from ·044 mm. to ·077 mm. The embryo, in common with that of the other species of the genus, is surrounded by a pyriform apparatus, the bulb of which immediately surrounding the embryo varies in diameter from ·022 mm. to ·029 mm. The bulb is prolonged into a pair of horns ·012 mm. in length, ending in a disc ·009 mm. in diameter.

Survey of the Genus *Moniezia* in relation to the New Species.

The species of Moniezia are divided into the following sub-groups:

A. Interproglottidal glands absent—*Alba Group.*

B. Interproglottidal glands present, arranged in a linear manner, not around blind sacs—*Planissima Group.*

C. Interproglottidal glands present, arranged around blind sacs—*Expansa Group.*

The new species, therefore, since it possesses linear interproglottidal glands, belongs to the Planissima Group. The members of this group, together with those species of Moniezia in whose description no mention of interproglottidal glands has been made, are detailed below. In each case, the characters which distinguish them from the new species are given.

M. amphibia (Linstow 1901) (1).

Interproglottidal glands not mentioned. Testes not confined to the posterior portion of the segment.

M. benedeni (Moniez 1879) (2).

In this species the interproglottidal glands are "linear but extremely indistinct (absent?)." The scolex is large and obtuse, measuring ·96 mm. broad and ·48 mm. long, *i.e.* twice as broad as long.

M. bipappilosa (Leidy 1875) (3).

The description of this species is too scanty even to warrant its inclusion in the genus Moniezia. It may, therefore, be disregarded.

M. caprae (Rudolphi 1810) (2).

The description is too scanty to allow acceptance as a true species. It may therefore be disregarded.

M. conjugens Schauter 1917 (4).

In this species the interproglottidal glands are "first linear, and later arranged around three blind sacs."

M. crassicollis Schauter 1917 (4).

Interproglottidal glands linear, but indistinct. Scolex 1·26 mm. broad. Strobila 45 cm. long.

M. crucigera (Nitzsch and Giebel 1866) (2).

The type specimen having been lost and the internal anatomy not having been described, there is no justification for including this species in the genus. It may therefore be disregarded.

M. latifrons Schauter 1917 (4).

Scolex very large, measuring 1·9 mm. in breadth.

M. neumanni Moniez 1891 (2).

In this species the testes are not confined to the posterior portion of the segment.

M. nullicollis Moniez 1891 (2).

Strobila 40 cm. long. Largest segments 8 mm. wide and 1 mm. long. Scolex 1·3 mm. in diameter. Interproglottidal glands not described.

M. parva Schauter 1917 (4).

Strobila 25–30 cms. long. Ripe segments 2·75 mm. broad, 1·25 mm. long.

M. pellucida Blei 1921 (4).

The testes in this species are arranged in two triangles.

M. planissima Stiles and Hassall 1893 (2).

In this species the testes are not confined to the posterior portion of the segment.

M. rugosa (Diesing 1850) (5).

This species has not been sufficiently described to make it certain that it belongs to the genus Moniezia. If it is, however, a true Moniezia, the fact that the testes are not confined to the posterior portion of the segment, would differentiate it from the new species.

M. triangularis Marotel 1912 (6).

In this species the testes are arranged in two triangles.

M. vogti (Moniez 1879) (2).

This species is not sufficiently described even to warrant its inclusion in the genus Moniezia. It may therefore be disregarded.

The above forms a complete list of the species of Moniezia if the following are added:

Alba Group.

M. alba (Perroncito 1879).

M. denticulata (Rudolphi 1810).

Expansa Group.

M. expansa (Rudolphi 1810).
M. minima Marotel 1912.
M. oblongiceps Stiles and Hassall 1893.
M. trigonophora Stiles and Hassall 1893.

It is clear, therefore, that the species is correctly regarded as new, and hitherto undescribed. I propose for it the name *Moniezia translucida* n.sp.

Specific Diagnosis of *Moniezia translucida* n.sp.

Adult strobila 150–200 cms. long, colourless and translucent in the anterior portion, yellowish in the mature portion. Scolex small, pear-shaped and always longer than broad; ·625–1·25 mm. long, ·496–·750 mm. broad, tapering gradually into neck. Suckers at four corners, running diagonally forwards. Neck long and slender, 2·38–4·63 mm. long, ·214–·496 mm. broad. Genital organs first visible 15–20 cms. from scolex. Segments always much broader than long, mature segments attain 12 mm. in breadth and 3 mm. in length. Interproglottidal glands linear and distinct. Genital pores double, situated in the anterior half of the lateral margin. On the right, vagina ventral and usually posterior, cirrus dorsal and usually anterior; on the left, vagina dorsal and usually anterior, cirrus ventral and usually posterior. Genital canals pass dorsal to longitudinal excretory canals and nerves. Testes arranged in a long narrow rectangle as numerous in the median line as elsewhere, but absent from the lateral fields, always confined to the posterior portion of the segment and always in a single dorsal layer. Uterine folds enter the lateral fields. Eggs ·044 mm.–·077 mm. in diameter. Bulb of pyriform apparatus ·022–·029 mm., horns ·012 mm., disc ·099 mm.

REFERENCES.

(1) von Linstow (1901). *Jenaische Zeitschr. f. Naturwiss.* v, 35, n.f.v. 28 (4), pp. 409–428; Plates XIII and XIV, figs. 1–34.
(2) Stiles, C. W. and Hassall, A. (1893). U.S. Dep. Agric., Bureau of Animal Industry, *Bull.* No. 4.
(3) Leidy, J. (1904). *Smithsonian Misc. Collec.* xlvi, No. 1477.
(4) Blei, R. (1921). Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Erste Abteilung. *Medizinisch-hygienische, Bakteriologie und tierische Parasitenkunde Originale,* Band 87, Heft 5. Gustav Fischer, Jena, December 17th.
(5) Lühe, M. (1895). *Arch. f. Naturg.* 1895, pp. 199–212.
(6) Marotel, G. (1913). 9th *congr. intern. Zool. Monaco,* 1913, pp. 662–663.

EXPLANATION OF PLATE XV

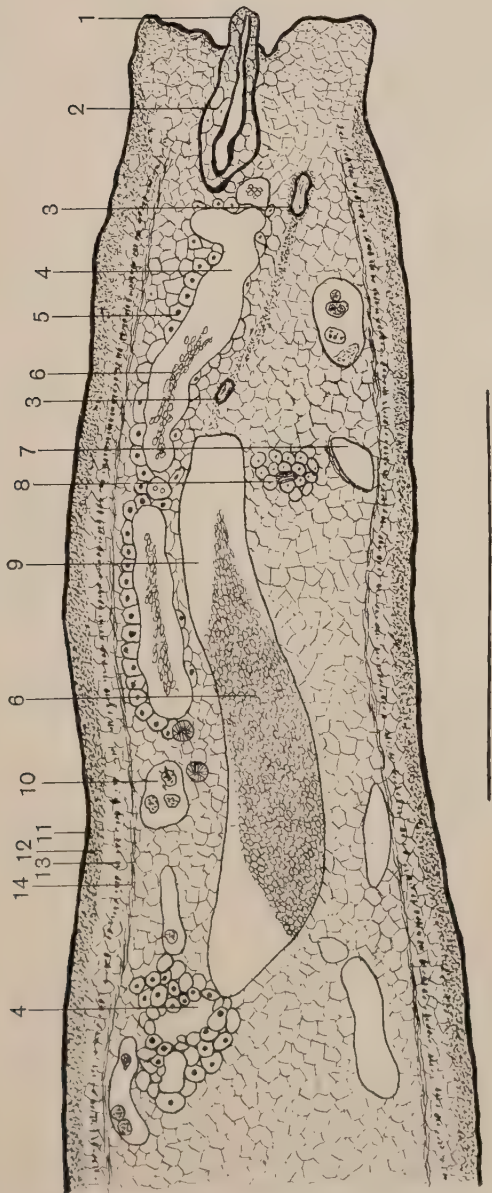
Fig. 1. Scolex.

Fig. 2. T.S. mature proglottis, right half. 1. Cirrus. 2. Cirrus pouch. 3. Vagina. 4. Vas deferens. 5. Prostatic cells. 6. Sperms. 7. Ventral excretory canal. 8. Dorsal excretory canal. 9. Receptaculum seminis. 10. Portion of uterus containing eggs. 11. Cuticle. 12. Sub-cuticular layer. 13. Longitudinal muscle layer. 14. Circular muscle layer.

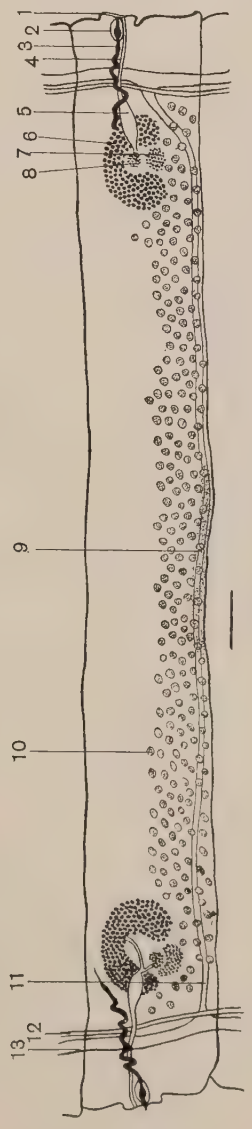
Fig. 3. Mature proglottis—pre-uterine stage. 1. Genital papilla. 2. Cirrus pouch. 3. Vas deferens. 4. Vagina. 5. Receptaculum seminis. 6. Ovary. 7. Vitelline gland. 8. Shell gland. 9. Interproglottidal gland. 10. Testes. 11. Transverse excretory canal. 12. Dorsal excretory canal. 13. Ventral excretory canal.

(*Received October 26th*, 1922.)

·5 mm.
Fig. 1.



·5 mm.
Fig. 2.



·5 mm.
Fig. 3.

JENKINS.—On a New Species of Moniezia from the Sheep. (pp. 276—286.)

STUDIES ON THE SEX-RATIO AND RELATED PHENOMENA

(3) NOTE ON THE INFLUENCE OF SIZE OF LITTER

By A. S. PARKES, B.A. (Cantab.).

(*Zoological Department, University of Manchester.*)

(With 1 Text-figure.)

CONTENTS.

	PAGE
Introductory	287
Objects	287
Previous investigations	288
Present data	289
Discussion	289
Summary	292

INTRODUCTORY.

The new data which are recorded in this paper have been compiled from the *Duroc Jersey Pig Herd Book*, Vol. 67. In this volume the number in the litter and the sexes at weaning time (presumably about seven weeks old) are given. Thus, if any of a litter die before weaning time the litter is not completely accounted for in the numbers of the sexes. This happens in a large percentage of cases, but for purposes of this investigation only those litters were included where the original sex constitution could be obtained.

I am much indebted to Miss M. Parkes for assistance in the analysis of the records.

OBJECTS.

It is hoped later to be able to complete a more detailed and analytical study on the subject; but in view of the time required for such work this preliminary note is put forward as a statement of what appears to the writer to be the nature of the problem of the possible influence of litter size on the sex-ratio, and as an attempt to correlate the existing work on the subject.

PREVIOUS INVESTIGATIONS.

Most of what little previous work there is has been done on animals which are normally polytocus. Some information exists, however, relating to the sex-ratio of multiple births in normally monotocus animals.

Pearl(7), for instance, notes a decline in the sex-ratio of twins and triplets in man.

That of twins was slightly below normal, while that of triplets was remarkably low at 54·8 males per 100 females.

For triplets in sheep Pearl found 215 females to 130 males, a sex-ratio of 60·6[1].

From these and similar figures it has been suggested that multiple births tend to produce an excess of females.

Newcomb(5), however, found nothing abnormal in the sex-ratio of dizygotic twins in man.

Wentworth(9), too, found that 146 cases of triplets in sheep gave 226 males and 212 females, a sex-ratio of 106·5, which is very near what is probably the normal ratio for sheep. Wentworth, also, working on pigs and dogs, found that there was nothing very unusual in the sex-ratio of multiple births.

This conclusion has also received support from King's(3) work on rats.

Parker and Bullard(6), working on rather different lines, give the following table showing that the size of the litter in pigs has little effect on the sex-ratio:

Table I.

Litter size and sex-ratio, from Parker and Bullard.

Number in litter	Males	Females	Sex-ratio
1	9	6	150
2	54	46	117·5
3	110	112	98·2
4	272	264	117
5	331	354	93·7
6	578	556	104
7	610	538	113·4
8	479	449	106·7
9	269	280	96·1
10	148	172	86
11	78	87	88·8
12	31	41	75·6
13	24	15	116
14	23	19	112·1
15	8	7	110·4
Totals	3024	2946	102·5

[1] All sex-ratios are calculated as males per 100 females.

These results show that the influence, if any, of the size of litter on the sex-ratio is small and is in any case erratic. Parker and Bullard came to negative conclusions from the results.

From Machens' excellent paper(4) the following data are taken for litter sizes at which ten or more litters were found:

Table II.

Litter size and sex-ratio, from Machens.

Size of litter	5	6	7	8	9	10	11	12	13	14
Sex-ratio	100	92·8	96·4	100	96·9	102·6	96·5	103·5	111·5	127·6

These again show an unfortunate incoherence.

It may be noted, however, that the sex-ratio of the total, 102·7, is remarkably similar to the result obtained by Parker and Bullard.

PRESENT DATA.

My own material tabulates out as under:

Table III.

Litter size and sex-ratio in Duroc Jerseys.

Size of litters	No. of litters	Males	Females	Total	Sex-ratio	% of total litters
4	26	59	45	104	131	2·7
5	54	155	115	270	134·8	5·5
6	110	338	322	660	105	11·4
7	186	689	613	1302	112	18·3
8	203	856	768	1624	111·5	20·9
9	175	783	792	1575	99	17·8
10	138	692	688	1380	100·6	14·2
11	63	355	338	693	105	6·4
12	27	180	144	324	124·5	2·8
Totals	982	4107	3825	7932	107·3	100

Litters of more than 12 and less than 4 are left out owing to being too few to give a reliable result.

The number of litters and the sex-ratio at each litter size are conveniently shown on a graph (Fig. 1).

DISCUSSION.

In all the cases mentioned above the importance of the results depends on the nature of the problem which the investigation was meant to elucidate. The early work on twinning was designed to throw light on the supposition that sex was determined by influence during gestation.

If this were so, twins, being developed under similar conditions, would be expected to show a greater proportion of unisexual births than would be expected by chance.

This old theory of sex-determination is hardly tenable now, and the

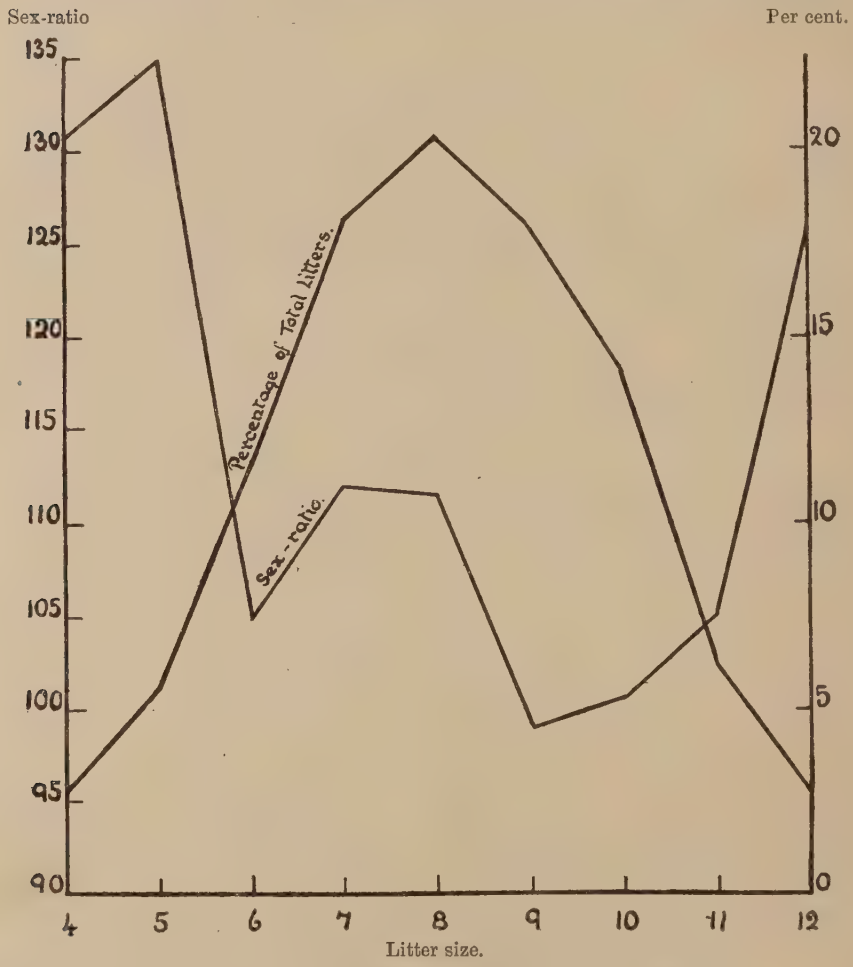


Fig. 1. Sex-ratio and percentage of total litters of each litter size.

later work on the sex-ratio and litter size seems to have been without any definite object.

The results mentioned above are far too indefinite to discuss theories of sex-determination upon, but viewed from other angles they are extremely interesting. From Wodsedalek's (10) work it seems eminently

probable that the modern tendency to locate sex-determination at fertilisation applies equally to the pig and other mammals, and this may be taken as a tentative basis for analysis of the above results.

Hammond(1) has shown that the number of full time foetuses in the pig is far less than the number of conceptions. This author found 49 atrophic foetuses to 267 normal ones, which is a mortality *in utero* of 15·5 %. Hence the size of a litter at birth is governed by two factors:

(1) The number of eggs fertilised.

(2) The mortality between conception and birth.

Now, on the current theory of sex-determination it is very hard to see how the number of eggs fertilised can affect the sex-ratio; but there is abundant evidence that the amount of foetal elimination between fertilisation and birth can affect the sex-ratio at birth. Schultz(8) for man, Jewell(2) for cattle, and my own observations on mice[1], have shown that in these three animals at least antenatal mortality falls preponderatingly upon the males.

We have now practically reduced the problem to whether or not the size of the litter can affect the amount of foetal elimination. In considering this it is essential to separate multiple births in animals which normally only produce one at a birth from multiple births in animals where they are the rule. Genital organs designed for one foetus will clearly have a greater difficulty in accommodating themselves to a number of foetuses than a normal uterus will in accommodating a litter larger than the average.

In man, male foetuses are larger than female ones, and thus male triplets would have less chance of arriving to full time than female triplets would have, and this fact probably goes a long way to accounting for the remarkable sex-ratio given for triplets by Pearl.

The case of the normal multiple pregnancies is very different. If the size of a litter can be the product of one or both of two factors, one of which affects the sex-ratio and one of which does not, it is not difficult to see that the results must be erratic, and in the case of the Duroc Jersey records the coherence found is more than might be expected.

The future line of work lies in investigating the relative importance of the two factors which govern the size of the litter, and their interrelation to the sex-ratio.

[1] As yet unpublished.

SUMMARY.

(1) The existing work on the influence of the litter size on the sex-ratio shows very erratic results, and the new work embodied in this paper is rather more coherent.

(2) It is clear, however, that multiple births in animals which are normally polytocus are somewhat different from multiple births in animals which are normally monotocus.

(3) Before any attempt can be made to arrive at the nature of the influence of the size of litter on the sex-ratio it is essential to consider the factors which govern the size of the litter. Hammond (1) has shown that the number of eggs fertilised and the amount of foetal elimination are the two main factors controlling the size of litter. The first of these factors can scarcely affect the sex-ratio, while the second, on the other hand, very well may have such an influence.

(4) In view of the complexity of the interaction of these two factors any great coherence in the resulting sex-ratio could hardly be expected.

LITERATURE.

(1) Hammond, J. (1921). *Journ. Agric. Sci.* xi.
(2) Jewell, F. M. (1921). *Biol. Bull. of Marine Biol. Lab. Woods' Hole*, xli.
(3) King, H. D., and Stotenburg, J. M. (1915). *Anat. Rec.* xi.
(4) Machens, A. (1915). *Berliner Tierärztliche Wochenschrift.* xxxi.
(5) Newcomb, S. (1904). Carnegie Instit. Wash. Pub. No. 11.
(6) Parker, G. H., and Bullard, C. (1913). *Proc. Am. Acad. Arts and Sci.* 48.
(7) Pearl, R. Quoted by Wentworth.
(8) Schultz, A. H. (1918). *Biol. Bull.* xxxiv.
(9) Wentworth, E. N. (1914). *Sci.* N.S. 39.
(10) Wodsedalek, J. E. (1913). *Biol. Bull.* xxv.

(*Received January 23rd*, 1923.)

REVIEW

Practical Zoology for Medical and Junior Students. By Prof. J. D. F. Gilchrist and C. von Bonde. E. and S. Livingstone, Edinburgh, 1922. Price: 15*s.* net.

Prof. Gilchrist's book tends at first glance to create a very favourable impression upon the reader. The publishers have certainly carried out their part of the work very well; the paper and the printing are attractive; the binding would have rejoiced the heart even of Chaucer's clerke of Oxenforde; the hundred and five illustrations, if lacking the originality and beauty of those of Kukenthal or Jammes, are at any rate clear, and do possess the merit of having their component parts *named* and not numbered nor lettered; the book is interleaved with blank paper, intended, although glazed, apparently for sketches.

Deeper acquaintance, however, induces a feeling of disappointment. In a book which is intended to compete against such a well established favourite as Marshall and Hurst's text-book, we have a right to expect outstanding originality in choice and treatment of types. On the contrary, we find only two types which are not dealt with by Marshall and Hurst, and these two, the Sponge and the Sea Anemone, are thoroughly described in Parker and Haswell. In any case, the inclusion of the latter type in a book intended for First Year Students is of doubtful value when it is made at the expense of the more typical and the metagenetic type of Coelenterate, such as *Obelia*. The value of the Earthworm as a type is similarly reduced by the failure to precede its description by an account of a more typical Chaetopod, *Nereis* for example. There is not a line of Embryology, not a line of Histology, and in a book catering primarily for South African Students the omission of such types as a Haemoflagellate, a Muscid Dipteron, a Grasshopper, and a Lizard, is inexplicable.

The dissection directions are feeble in the extreme. A book on Practical Zoology, written in 1922, should describe methods which were unknown possibly to compilers of previous manuals. Fifteen shillings is a high price to pay for information that could be better obtained verbally from one's grandfather. There are, for example, at least two methods of exposing the Afferent Branchial System of the Elasmobranch which replace the notoriously inadequate method of Marshall and Hurst; there

is a method of exposing the Sympathetic Nervous system of the Frog by dissection from the dorsal side; there is the tap-stream method of obtaining the alimentary canal and nerve cord of the Cockroach intact. Are these and many other points unknown to the compilers of this practical handbook, or are they unworthy of consideration?

On the whole, we cannot congratulate Prof. Gilchrist and his collaborator upon the book. A more judicious selection of types and a more careful attention to details of technique would have rendered such a work attractive and valuable to students of all grades, but as it is, in spite of many admirable features, it runs a risk of becoming regarded merely as an emasculated version of Marshall and Hurst.

R. A. W.